INFLUENCE OF DIETARY FACTORS ON CALCIUM METABOLISM AND INCIDENCE OF PARTURIENT PARESIS IN DAIRY COWS

BY

CHANGZHENG WANG

A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1990

To my parents

ACKNOWLEDGEMENTS

This dissertation contains the dedication, knowledge and work of many individuals. I would like to express my sincere gratitude to Dr. David K. Beede for the opportunity to work with him, for his encouragement, support and guidance throughout my graduate program. I also would like to thank Dr. Alfred M. Merritt, II for his valuable suggestions and generous help with various phases of this work. I thank Dr. Charles J. Wilcox, Dr. William W. Thatcher and Dr. Lynn B. Bailey as members of my supervisory committee for their guidance and advice.

Acknowledgements are extended to Dr. K. C. Bachman, Dr. Barbara Brewer for generously allowing me to use their equipment. Special thanks go to Dr. G. A. Donovan and Dr. L. F. Archbald for their valuable contribution to this work and for their advice. I also wish to thank Dr. C. R. Staples, Dr. Barney Harris, Jr. for their advice and help during my stay here.

I also would like to thank Joyce Hayen and Estelle Hirchert for their friendship and endless help and support in the lab, Mr. Dale Hissem for his friendship and generous help with the experiments conducted at the research farm, and Margaret Bates for her expert assistance with the preparation

of the Dairy Research Unit for taking care of the experimental cows.

I would like to give special thanks to Mr. Don Bennink and Mr. Don Strong for the opportunity to conduct the field study at North Florida Holsteins Dairy and for generous help offered by them and their staff.

I would like to express my sincere appreciation to Bill Sanchez for his friendship, help with experimental work, valuable advice and suggestions on various other matters. I also would like to thank Francois Elvinger, Craig Thomas, David Wilfond, Lynda Ferguson, Devin Dorminey, Ted Ruiz, Rafael Umana, R. Luzbel de la Sota, Matt Lucy, Doug McCullough, Lee Chul-Young, Carlos Garcia, Dr. Scott Lough, Dr. Lokinga Badinga, Dr. Steve Emmanual, Dr. Anita O'Connor, Mark McGuire and Mike McGlothlen for their friendship and help with my experiments.

I am indebted to my parents and family for putting me through school under great difficulties and for their love and support throughout my studies. More importantly, my wife, Lily, with her love, encouragement and support, made it possible for me to complete this work.

TABLE OF CONTENTS

LIST OF TABLES

TABLES

TABLES

LIST OF FIGURES

FIGURES

Abstract of Dissertation Presented to the Graduate School of the University of Florida in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

INFLUENCE OF DIETARY FACTORS ON CALCIUM METABOLISM AND INCIDENCE OF PARTURIENT PARESIS IN DAIRY COWS

By

CHANGZHENG WANG

December, 1990

Chairman: Dr. David K. Beede
Major Department: Animal Science

Effects of several dietary factors on calcium metabolism and incidence of parturient paresis were investigated in four experiments. In experiment one, a replicated 3 X 3 Latin square design was used with six nonlactating and nonpregnant Jersey cows. Increasing dietary protein intake from 11% to 19% DM induced a mild acidosis, but did not affect the apparent balances of Ca, P or Mg. Plasma Ca concentrations and responses to Na_2EDTA infusion were not affected by dietary protein intake. In experiment two, eight nonlactating and nonpregnant Jersey cows were used in a switch-over design. Supplementation of NH_4Cl and $(NH_4)_2SO_4$ decreased blood and urine pH, but increased urine titratable acidity, ammonium and net acid concentrations. Compared with cows fed a control diet, cows fed the acidogenic salts had a higher blood concentration of ionized Ca, and their blood Ca concentrations recovered faster after Na_2EDTA infusion ceased. In experiment three, eight nonlactating and nonpregnant Holstein cows were used in a switch-over experiment with three periods of 28 d

each. The normal Mg diet was supplemented with NH_4Cl and $(NH_4)_2SO_4$, whereas the high Mg diet had NH_4Cl and $MgSO_4$ to provide equivalent amount of S but additional Mg. Cows fed the high Mg diet had slightly higher blood pH and plasma concentrations of total Ca but lower plasma concentrations of P. The responses to Na_2EDTA infusion were similar for cows fed either the normal Mg or the high Mg diet. The $MgSO_4$ had slightly less effect on acid-base status than $(NH_4)_2SO_4$. In experiment four, a total of 510 cows were assigned randomly to a typical diet for late gestation cows or to a diet with negative cation-anion difference 21 d before expected parturition. The diet with negative cation-anion difference increased serum ionized Ca concentrations and reduced incidence of parturient paresis and hypocalcemia, but did not affect incidence of other diseases, nor overall production. Cows of the second, third and fourth parity produced more milk when fed the diet with negative cation-anion difference.

In conclusion, dietary protein intake in dairy cows did not affect Ca metabolism. Supplementation of ammonium salts increased cows' ability to maintain the blood Ca concentration close to normal when the demand for Ca was increased suddenly by Na_2EDTA infusion. Substitution of $(NH_4)_2SO_4$ by $MgSO_4$ did not affect responses to Na_2EDTA infusion, but resulted in smaller effects on acid-base status. Diets with negative cation-anion difference (with NH_4Cl, $(NH_4)_2SO_4$ and $MgSO_4$) improved Ca metabolism and reduced incidence of parturient paresis.

CHAPTER 1
INTRODUCTION

Parturient paresis (PP), commonly known as milk fever, is one of the most common production diseases associated with parturition and early lactation in dairy cows. Typical clinical symptoms of PP are changes in neuromuscular tone, ranging from fine tremors in the early stages to paresis and recumbency. Initial restlessness is followed by progressive somnolence and finally coma. In clinical cases, most untreated cows die within 1 or 2 d (Hibbs, 1950).

Incidence of PP varies greatly from herd to herd, and nationwide is approximately 5% of all milking cows (Horst, 1986). First calf heifers are affected rarely by PP, but there is increased incidence of PP with increasing parity from the second to some plateau around fourth to sixth lactation (Curtis et al., 1984). Among dairy breeds, Jerseys are more susceptible than other breeds (Curtis et al., 1970). The incidence and severity of the disease increases as production increases (Moodie, 1965). In the last 30 yr the average milk production/cow has increased significantly in the United States. At the same time the nutritional and management spectrum, over which these animals can maintain their metabolic homeostasis, has narrowed dramatically (Littledike et al., 1981). Thus, the incidence of milk fever has tended

to increase (Jorgensen, 1974). Treatment of the disease can be inconvenient and costly, and the disease may reduce the productive life span of dairy cows by 3.4 yr (Payne, 1968). Cows with PP are three to nine times more likely to contract other postpartum disorders, such as dystocia, retained fetal placenta, ketosis and mastitis (Curtis et al., 1983). Therefore, this disease is of considerable economic importance.

Little and Wright (1925) were the first to establish that the major change in the blood of cows with PP was a decrease in the concentration of Ca, and the severity of the disease was related inversely to the plasma Ca concentration. Although hypocalcemia could occur without symptoms of milk fever, all cases of true PP are associated with a considerable degree of hypocalcemia at some stage (Moodie, 1965). Consequently, hypocalcemia and PP tended to be considered synonymous (Moodie, 1965).

Numerous methods for prevention of PP have been examined. Manipulation of dietary Ca intake prepartum and feeding or injecting large doses of vitamin D or vitamin D metabolites have been considered promising in prevention of PP (Boda and Cole, 1954; Boda, 1956; Goings et al., 1974; Care et al., 1980). Other studies suggest that diets with negative cation-anion difference reduce incidence of PP (Ender et al., 1962; Dishington, 1975; Dishington and Bjornstad, 1982; Block, 1984). In this dissertation, Ca metabolism and the dietary

factors potentially influencing Ca metabolism will be examined to search for new methods and improvement on the existing measures for the prevention of PP. One method with potential for successful practical application will be evaluated on a commercial dairy herd.

CHAPTER 2
LITERATURE REVIEW

General Review of Calcium Metabolism

An overall scheme of Ca metabolism has been presented by Horst (1986). Calcium in the plasma is exchangeable with a pool of Ca about 35 times larger than the amount circulating in the blood (Ramberg et al., 1975). Calcium is supplied to this pool through intestinal absorption and bone resorption. Calcium exits from this pool into feces, urine and bone in a nonpregnant, nonlactating cow. Calcium deposition into the fetus and secretion into milk constitute additional Ca losses for pregnant and lactating cows. The maintenance of normal plasma Ca concentrations depends upon the balance between total inflows and total outflows of Ca associated with this pool (Ramberg et al., 1975).

An imbalance between the inflows to and outflows from the exchangeable Ca pool would change the plasma Ca concentration. Fecal Ca includes unabsorbed Ca and endogenous Ca excreted into feces. The amount of unabsorbed Ca is dependent on the absorption efficiency, but the endogenous fecal Ca is relatively constant, increasing slightly with aging (Hansard et al., 1954). Urine Ca normally accounts for about 1% of total Ca excretion because bovine kidneys are highly efficient in conserving Ca (Moodie, 1960). Amount of Ca excreted into

milk could be very large, depending on the milk yield. Approximately 2.5 g Ca is extracted from the blood/kg colostrum produced. This approximates the total amount of Ca present in the blood at any given time (Horst, 1986). Initiation of lactation provides a great challenge to Ca homeostasis of dairy cows at parturition. Early studies suspected that excessive loss of Ca into the colostrum might be responsible for the severe hypocalcemia in cows suffering from PP. However, the major limiting factor in the maintenance of normal blood Ca concentrations appears to be the amount of Ca inflow to the exchangeable Ca pool. The total Ca inflow is dependent essentially on Ca absorbed from the intestine and Ca mobilized from bones (Horst, 1986). Thus, Ca absorption and bone resorption will be discussed in more detail.

Calcium Absorption

Calcium absorption occurs mainly in the upper small intestine (duodenum), even though there is some evidence that Ca may be absorbed in the forestomachs of ruminants (Timet and Stojevic, 1982). The absorption process involves entry at the brush border, transport through the cytoplasm and extrusion at the basolateral membrane of the enterocytes (Bronner et al., 1983). Cytosolic Ca concentration is in the micromolar range and the transmembrane electrical potential is negative in the cell interior. Consequently, the electrochemical potential

favors the diffusion of Ca into the cells and limits the transport of Ca across the basolateral membrane (Favus, 1985). Calcium enters the epithelial cells across the microvillus membrane by passive diffusion or facilitated movement, whereas it is pumped across the basolateral membrane (Murer and Hildman 1981). Calcium also may pass the basolateral membrane by diffusion (Wasserman and Taylor, 1969). This diffusion process may by-pass the cellular electrochemical potential via a paracellular pathway (Favus, 1985). Wasserman and Kallfelz (1962) showed that Ca influx was not saturable at concentrations up to 135 mM. It was suggested later that these data were compatible with an active saturable component and a simple diffusion system for Ca absorption (Wasserman and Taylor, 1969). In vitro work on Ca influx in rat duodenal slices also has indicated that Ca movement into the cell is by a combination of a saturable component and a diffusion component (Papworth and Patrick, 1970). Although the saturable, active Ca absorption process is dominating when the Ca concentration is low, significant amounts of Ca may be absorbed by passive diffusion when the Ca concentration is high on the luminal side of the intestine (Harrison and Harrison, 1960, 1965).

The enzyme Ca-ATPase was found on the basolateral membrane in the intestine of rats (Ghijsen et al., 1982). The activity of this enzyme correlates well with the uptake of Ca by the basolateral membrane vesicles and Na is required for

the transport of Ca (Ghijsen et al., 1982). According to the model of Schachter and Kowarski (1985), Ca enters across the luminal membrane via a vitamin D-dependent diffusion process and then is pumped out across the basolateral membrane via a Ca-ATPase. A Ca-Na exchange mechanism may be involved also. Activity of Ca-ATPase appears to be highest in the cells at the mid-villus, but least active in the youngest cells at the crypt of the intestinal epithelium (Van Corven and Van Os, 1984). Once inside the cell, Ca may have to be bound to maintain the low cellular ionic Ca concentration and prevent potential toxic effects to the cell. It has been shown that golgi apparatus and mitochondria actively take up Ca (Roche et al., 1984). How this relates to the entry and extrusion of Ca is not well understood currently.

Parathyroid hormone (PTH) is a major hormone regulating Ca metabolism. It stimulates Ca absorption, but vitamin D_3 is necessary for its action (Dowdle et al., 1960). Olson et al. (1972) also showed that parathyroid hormone did not affect Ca absorption in vitamin D deficient rats. It appears that parathyroid hormone does not affect Ca absorption directly but indirectly by affecting the metabolism of vitamin D. Vitamin D is converted to 25-hydroxyvitamin D ($25\text{-}OHD_3$) in the liver (Deluca, 1981). The $25\text{-}OHD_3$ then is converted to several metabolites, of which 1,25 dihydroxyvitamin D ($1,25\text{-}(OH)_2D_3$) is the most active form biologically (Horst and Reinhart, 1983). The $25\text{-}OHD_3$ is converted to $1,25\text{-}(OH)_2D_3$ by kidney 1a-

hydroxylase enzyme, which is stimulated by parathyroid hormone (Garabedian et al., 1972). After thyroparathyroidectomy, lactating goats developed hypocalcemia and had low concentration of plasma $1,25\text{-}(HO)_2D_3$; infusion of PTH (3 ng/kg/min) corrected the hyocalcemia and markedly raised plasma concentrations of $1,25\text{-}(OH)_2D_3$ (Hove et al., 1984). This indicates that PTH stimulates the synthesis of $1,25\text{-}(OH)_2D_3$ under hypocalcemia. In the same study, when $CaCl_2$ was infused, together with PTH, plasma concentrations of $1,25\text{-}(OH)_2D_3$ were not increased. It appears that PTH plays a lesser role than the plasma Ca concentration on plasma concentrations of $1,25\text{-}(OH)_2D_3$ in the ruminants when potentially conflicting signals, such as hypercalcemia and high PTH concentrations, are present simultaneously (Hove et al., 1984).

The $1,25\text{-}(OH)_2D_3$ is a major hormonal regulator of intestinal Ca absorption. It affects all phases of the transport system (Wasserman et al., 1984). The entry across the brush border, translocation through the cytosolic compartment and extrusion across the basolateral membrane are all increased by the administration of vitamin D. Like other steroid hormones, at least part of its mechanism of action involves new protein synthesis (Bikle et al., 1984). The binding of $1,25\text{-}(OH)_2D_3$ to specific receptors located in the enterocytes is followed by nuclear localization of the hormone-receptor complex and subsequent appearance of newly synthesized proteins, such as Ca-binding protein. Although

Ca-binding protein is certain to increase Ca absorption, the actual mechanism of its action is not well defined. Because 90% of this soluble protein is in the cytosol, one of its action may be the binding of Ca, preventing the concentration of ionic Ca inside the cell from increasing to toxic level. It may also function at the brush border surface or at the basolateral membrane (Lawson, 1978).

Other effects of 1,25-$(OH)_2D_3$ have been suggested also. Bikle et al. (1978) observed that cycloheximide failed to block 1,25-$(OH)_2D_3$ induced Ca transport across the chick duodenum despite its blocking the stimulating effect on the alkaline phosphatase activity and Ca-binding protein. It was found that 1,25-$(OH)_2D_3$ increased calmodulin content in the brush border membrane vesicles in parallel with the increased ability of the vesicles to accumulate Ca (Bikle et al., 1984). It seems that 1,25-$(OH)_2D_3$ increases the binding of calmodulin to specific membrane proteins. Perhaps one (or more) of these calmodulin binding proteins is the calcium channel in the brush border membrane, which in the presence of calmodulin permits a greater influx of Ca into the intestinal cells.

Rasmussen et al. (1982) observed that two major changes in the membrane lipid composition induced by vitamin D treatment were an increase in the content of phospholipids per mg of membrane protein and a specific change in the fatty acid profile found in the phosphatidyl choline fraction. It was suggested that 1,25-$(OH)_2D_3$ also may affect the fluidity of the

brush border membrane by inducing alterations in the phospholipids, resulting in an increase in the permeability of the membrane to Ca (Matsumoto et al., 1981).

Bone Resorption

Over 99% of the total Ca in the body is stored in bones. Nearly all trabecular bone and 7 to 17% cortical bone Ca may be mobilized to maintain Ca homeostasis (Luick, 1957). Months or perhaps years are needed to deplete skeletal Ca reserves (Ramberg et al., 1975).

Resorption of bone involves release of bone mineral and degradation of bone matrix. Most bone resorption is cell-mediated (Mundy and Raisz, 1981). Mineral may also be exchanged between bone and extracellular fluid without removal or degradation of the bone matrix (Bohatirchuk, 1966). Bone is constantly being formed and resorbed. This continual modeling and remodeling of bone is necessary for normal skeletal growth, repair of fracture and maintenance of blood Ca concentrations. Nordin (1976) suggested that changes in bone resorption and formation may be more important in more long term control of Ca homeostasis. Others suggested that the rapid exchange of mineral without the changes in bone matrix control minute-to-minute regulation of serum Ca concentration (Raisz, 1976).

Bone has three major types of cells: osteoblasts, osteocytes and osteoclasts (Revell, 1986). Osteoclasts are

responsible mostly for the cell-mediated bone resorption. The number and activities of osteoclasts are stimulated by PTH (Raisz, 1965), vitamin D metabolites (Trummel et al., 1969), but are inhibited by calcitonin (Raisz and Niemann, 1968).

Most evidence suggests that the formation of osteoclasts is affected by a cellular cascade involving the transformation of pluripotent hematopoietic stem cells to circulating monocytes to tissue macrophages with ultimate fusion to form multi-nucleated osteoclasts (Louitit and Nisbet, 1982). In support of this hypothesis, monocytes and macrophages were found capable of resorbing deactivated bone chips in vitro (Kichura et al., 1982). Key et al. (1984) demonstrated that in patients with osteopetrosis monocytes lose their ability to resorb bone in vitro. Supplementation with pharmacological doses of $1,25\text{-}(OH)_2D_3$ improved the ability of the patients' monocytes to resorb bone in vitro. Moreover, the presence of $1,25\text{-}(OH)_2D_3$ enhanced the maturation of monocytes to macrophages, which are thought to be the direct precursor of osteoclasts. Parathyroid hormone and $1,25\text{-}(OH)_2D_3$ may regulate bone resorption by affecting these processes.

Osteoblasts do, but osteoclasts do not have receptors for PTH (Marks, 1983). Parathyroid hormone does not stimulate osteoclasts directly (Rodan and Martin, 1981). However, culture media of osteoblasts stimulated by PTH increased osteoclast activity (Perry et al., 1987), suggesting that PTH could stimulate osteoblasts to synthesize certain bone

resorptive factors to affect osteoclasts. A protein with relatively high molecular weight may be involved (Perry et al., 1987). Calcitonin appears to inhibit bone resorption by acting on osteoclasts directly (Rodan and Martin, 1981). Other factors that affect the activities of the osteoclasts directly or the regulatory hormones also may affect Ca mobilization from bone.

Causes of Parturient Paresis

Cows that develop PP are unable to meet the sudden increased demand for Ca, which is brought about by the sudden loss of this mineral from the blood for the formation of colostrum at parturition. A cow producing 25 kg milk/d will have to replace her total blood Ca about every hour (Horst, 1986). Initiation of lactation is associated closely with hypocalcemia at parturition. This is supported by Niedermeier et al. (1949), who found little change in plasma Ca concentration at parturition in mastectomized cows. Alternating periods of milking for 4 d and nonmilking for 3 d in postpartum cows also showed that initiation of lactation induced a 3 to 4 d hypocalcemia (Littledike et al., 1983). These results demonstrate that initiation of lactation represents a great challenge to the cow'ability to maintain Ca homeostasis. However, Ca output into colostrum in cows with milk fever does not differ from that of nonparetic parturient cows (Hibbs et al., 1951). No difference was found between

paretic parturient cows and normal parturient cows in the rate of mammary blood flow or arteriovenous differences of Ca concentrations in mammary blood (Kronfeld and Ramberg, 1971). These suggest that severe hypocalcemia at parturition is not caused by excess outflow of Ca from blood Ca pool into milk, but most likely is caused by a reduced inflow of Ca from the gut or bone.

Decreased absorption of Ca from intestine, either due to the lack of Ca in the intestine or to the low efficiency of Ca absorption, lead to hypocalcemia. This was shown in lactating cows fed high Ca diets. When the dietary Ca concentration was decreased suddenly or the diet intake was restricted, the cows developed hypocalcemia (Hove and Hilde, 1984). Fasting or inhibiting motility of the gut by injection of hyoscinehydrobromide also induced hypocalcemia (Robertson et al., 1960; Moodie and Robertson, 1962). Inappetence and intestinal hypomotility frequently occur in parturient cows due to hormonal effects or the stress of parturition (Moodie and Robertson, 1961), especially in old cows (Marquardt et al., 1977). Thus, the cow could be more susceptible to milk fever if she is dependent mainly on Ca absorption to maintain her Ca homeostasis at parturition.

Decreased mobilization of Ca from bone also resulted in hypocalcemia. This were supported by the induction of PP with the administration of disodiumethane-1-hydroxy-1,1-diphosphate (EHDP), which is a bone resorption inhibitor (Yarrington et

al., 1976). Cows with PP had lower plasma and urine concentrations of hydroxyproline, also indicating a reduced rate of bone resorption (Black and Capen, 1973).

Failure to mobilize Ca from bone may arise from excessive secretion of calcitonin. Although Capen and Young (1967) indicated that thyroid glands of cows with parturient paresis contained less calcitonin than normal parturient cows, probably due to excessive secretion, it is difficult to accept this hypothesis due to the lack of a known stimulus for such an excessive secretion of calcitonin. Only an increase in plasma Ca concentration stimulates calcitonin secretion, but hypercalcemia prior to parturition has not been observed (Care et al., 1968). Furthermore, Forslund et al. (1980) found no difference in plasma concentrations of calcitonin between normal cows and cows with milk fever. In fact, cows with PP had lower blood concentrations of calcitonin than normal cows (Mayer et al., 1975). Thus, the reduced calcitonin content in the thyroid gland may suggest that cows with PP have a lower calcitonin secretion rate rather than hyperactivity.

A deficiency of parathyroid hormone (PTH), as first suggested by Dryerre and Greig (1925), may be responsible for low resorption rate of Ca from bone. However, results conflicting with this hypothesis were reported. Ramberg et al. (1966) found no difference in PTH secretion between normal cows and cows with PP. Even higher concentrations of PTH were observed in cows with PP indicating PTH secretion was not

impaired (Mayer, 1969). Concentrations of 1,25-$(OH)_2D_3$ were similar among cows with PP and normal cows (Horst, 1978). However, the target organs of PTH and 1,25-$(OH)_2D_3$, especially, intestine and bone, may be unresponsive temporarily in cows with PP. Administration of parathyroid extracts within 2 h postpartum had no effects on the incidence or severity of PP (Jackson et al., 1962).

Microradiographic and histological evaluation of cortical and trabecular bone taken from PP-prone cows suggested that the inability to mobilize bone resulted from an impairment of osteoclast function (Yarrington et al., 1977). This was in contrast to the active osteoclast-mediated bone resorption occurring in normal animals (Rowland et al. 1972). Failure of osteoclastic response in milk fever is quite similar to that observed in congenital osteopetrosis, where bone resorption is defective also due to abnormal osteoclast function (Key et al., 1984). Parturient paresis, therefore, may represent an acute nutritional osteopetrosis similar to that in bulls fed high-Ca diets (Krook et al., 1971). The impaired function of osteoclasts may result from either a lack of or malfunction in the precursor cells (monocytes), and possibly some interruption of conversion from the precursor cells to osteoclasts. Horst and Reinhardt (1983) suggest that PP could result from defects in the interation between 1,25-$(OH)_2D_3$ and its receptors in the target organs.

The relative importance of depressed absorption of Ca from the gut and depressed mobilization of Ca from bone as causes of hypocalcemia is not well defined. In some studies where the role of depressed Ca absorption was emphasized (Moodie and Robertson, 1962; Robertson et al., 1960), mobilization rate of Ca from bone was not examined. In other studies where the role of depressed mobilization rate of Ca from bone was emphasized (Yarrington et al., 1976; Yarrington et al., 1977), Ca absorption from the intestine also played a role. It seems that all combinations of different rates of Ca mobilization from bone and different amount of Ca absorbed from the gut (depending on the efficiency of absorption, and Ca available for absorption in the gut) are possible under practical conditions. A high rate of Ca mobilization from bone may compensate for a temporary disruption of Ca absorption, and vice versa, so the total inflow of Ca into the exchangeable Ca pool is maintained close to normal. Parturient paresis occurs when Ca mobilization or Ca absorption is depressed so serverely that the total inflow of Ca into the exchangeable Ca pool is reduced.

A hypothesis proposed by Craige and Stoll (1947) suggested that hypocalcemia is a characteristic symptom, but a parturient alkalosis might be the fundamental physiological defect in cows with milk fever. Coexistence of acetonemia may be a biological attempt to correct a parturient alkalosis. This theory essentially was ignored for a very long period.

Acid-base balance is associated closely with Ca metabolism (Barzel, 1981). Reynolds (1971) observed that a respiratory alkalosis developed in cows near parturition, probably as a result of hyperventilation. The alkalosis became more severe as the cow got closer to calving. It is possible that alkalosis impairs the function or the formation of osteoclast cells and reduces the responsiveness of bone to parathyroid hormone and $1,25\text{-}(OH)_2D_3$. Alkalosis also may lead to defects in the hormone and receptor interaction suggested by Horst and Reinhardt, 1983. In light of the findings that acidogenic diets fed prepartum prevent milk fever (Dishington, 1975), the value of this hypothesis needs to be reassessed.

Calcium Intake on Calcium metabolism and Prevention of Parturient Paresis

Dietary Ca intake during the dry period affects both Ca absorption and Ca mobilization from bones around parturition (Ramberg et al., 1984). In cows fed diets with high Ca content, Ca inflow during the first few days following parturition was dependent mainly on increased absorption of Ca from the gut. However, in cows fed low Ca diets, Ca mobilized from bone is the major inflow to the Ca pool.

A low Ca intake (8 g/d) tended to increase bone mobilization prepartum and increased bone mobilization significantly peripartum, as indicated by increased plasma concentrations of hydroxyproline (Green et al., 1981). Yarrington et al. (1977) provided more substantial results

supporting this hypothesis. Following a 4 h EDTA infusion given 10 d prepartum, the recovery rate of serum Ca concentration was greater in cows with low Ca intake (9.5 g/d) than in those fed 25g Ca daily for 70 d prepartum. The specific activity of intestinal and renal Ca-binding protein, percentage of bone surfaces undergoing resorption and the number of cortical resorptive spaces in the bone were higher 1 d postpartum for cows fed the low Ca diet than those fed the control diet. Urinary hydroxyproline excretion at 1 d postpartum was increased to 553% above the prepartum values for cows with low Ca intake compared with 349% for cows with normal Ca intake. Histological and ultrastructural assessments of the thyroid and parathyroid glands of these animals indicated that Ca homeostasis in cows fed a low Ca diet prepartum was directly under the control of PTH-mediated bone resorption with the approaching of parturition. It appears that the increase in the rate of Ca removal from bone at parturition is more rapid in cows conditioned to have a greater rate of Ca removal from bone before parturition. It was suggested that, as in many other physiological systems, previous exercise of the bone resorbing mechanisms resulted in a more favorable response to a sudden metabolic stress, such as the initiation of lactation (Ramberg et al., 1975).

Avoiding excessive dietary Ca intake prepartum for a certain period of time has been considered an effective means of preventing milk fever (Boda and Cole, 1954, 1956; Boda,

1956; Goings, et al., 1974 and Wiggers et al. 1975). This approach appears to take advantage of the cows's inherent Ca homeostatic mechanisms and results in stimulating PTH and ultimately 1, 25-$(OH)_2D_3$ synthesis, which acts to stimulate bone resorption and intestinal Ca absorption. Ramberg et al. (1984) showed that cows depended almost solely on bone Ca resorption to maintain its Ca homeostasis when dietary Ca is as low as 10 to 15 g/d. Yarrington et al. (1977) reported relative dependency on dietary Ca to maintain Ca homeostasis in cows fed 25 g Ca/d, but cows fed 8 g Ca/d were dependent on bone mobilization to maintain their Ca homeostasis.

Incidence of milk fever was reduced when Ca intake was below 15 to 25 g/d (Boda and Cole, 1954; Boda, 1956 and Wiggers et al. 1975). Such low rates of Ca intake are difficult to achieve with most conventional dietary regimens (Jonsson, 1978). Many forages typically are quite high in Ca content (NRC, 1989). One may have to restrict the forage intake or the total dietary intake. This may predispose the cow to digestive disorders and ketosis, and reduce the capability of the cow to reach her production potential postpartum (Verdaris and Evans, 1974). Additionally, when not enough attention is given to the amount of feed offered and the number of cows fed in a group, total ration and total Ca intakes may be greater than prescribed.

European workers were unsuccessful in preventing milk fever by lowering the Ca intake from 150 g/d to 30 to 40 g/d

(Jonsson and Pherson, 1975). Van de Braak et al. (1986a) also showed that risk of PP in cows fed 84 g Ca/d or 15g Ca/d was similar. Similarly Oetzel et al. (1988) did not find any difference in incidence of milk fever and hypocalcemia between cows fed 53 g Ca/d and those fed 105 g/d. This indicates that factors other than Ca intake may be involved. Van de Braak et al. (1986b) suggested that keeping feed intake of dry cows close to maintenance level might help to prevent PP. In previous studies where preventive effects of low Ca intake were suggested (Boda and Cole, 1954; Boda, 1956), cows on the low Ca diets had lower feed intake than cows on the high Ca diets. The low feed intake might contribute to the preventive effects of the low Ca diets.

Increasing Ca intake immediately before calving appeared to have some beneficial effects. Drenching with 150 g of $CaCl_2$ daily on the day before calving and the following 4 d reduced the incidence of milk fever from 46% to 23% (Jonsson, 1978). A $CaCl_2$ gel given to cows orally at rates of 100, 115 or 232 g/d for 5 to 10 d around parturition reduced milk fever incidence from 50% to 10% (Jonsson, 1978). Pickard (1975) suggested increasing Ca intake to 100 g/d near calving from 50 g/d before. Feeding 150 g bone meal/d for 2 d before to 1 d after calving did not prevent milk fever (Witter et al., 1985), indicating that increasing Ca intake immediately around parturition was not always effective either.

Vitamin D and Its Metabolites on Calcium Metabolism and Prevention of Parturient Paresis

Theoretically any defect in the metabolism of vitamin D could lead to hypocalcemia due to its important role in Ca homeostasis. Horst et al. (1978) showed that plasma concentrations of 1,25-$(OH)_2D_3$ in cows with PP were even higher than those in normal cows, indicating no problem in the conversion of vitamin D to its biologically active form. Horst and Reinhart (1983) proposed that there might be abnormalities in the interaction of 1,25-$(OH)_2D_3$ and its receptors in the target tissues. They further suggested that excessive glucocorticoid at parturition may down regulate 1,25-$(OH)_2D_3$ receptors, reducing Ca absorption from the intestine. Other factors, hormonal or dietary also may affect the 1,25-$(OH)_2D_3$ receptors. Further studies are needed to provide specific information.

Treatments including large oral doses of vitamin D (Hibbs and Pounden, 1955; Capen et al., 1968), parental administration of the active vitamin D metabolite, 25-$(OH)D_3$ (Olson et al., 1973), 1a-hydroxyvitamin D (1a-$(OH)D_3$) (Marquardt et al., 1974; Sachs et al., 1977), 1,25-$(OH)_2D_3$ (Gast et al., 1979) were used with varying degrees of success in PP prevention. One problem with using these compounds is the inhibition of the kidney 1a-hydroxylase. Littledike and Horst (1982) found that many of the cows treated with vitamin D or 1a-$(OH)D_3$ derivatives prepartum showed hypocalcemia and clinical signs of milk fever at 10 to 14 d postpartum. In the

control group plasma 1,25-$(OH)_2D_3$ was related inversely to plasma Ca concentrations (r=-.82), while in the treated group plasma 1,25-$(OH)_2D_3$ did not increase in response to hypocalcemia (r=.37). Cows in the treated group became dependent on the exogenous 1,25-$(OH)_2D_3$ to reverse their hypocalcemia.

Vitamin D metabolites mainly stimulate Ca absorption from the gut. Administration of these substances prepartum may make cows more dependent on Ca absorption to maintain Ca homeostasis. Thus, bone resorption is depressed and the response to the sudden withdrawal of Ca becomes inadequate. This seems to be justified by the findings that 1,25-$(OH)_2D_3$ treated cows suffered serious hypocalcemia at parturition and once or twice postpartum (Horst, 1986). Plasma concentrations of 1,25-$(OH)_2D_3$ appeared to change cyclically postpartum with the lowest concentration occurring every 8 to 10 d in some cows (Hove, 1986). Treatment with 1,25-$(OH)_2D_3$ may exaggerate these cyclic changes.

The activity of the parathyroid glands may be depressed (Hibbs et al., 1951). Capen et al. (1968) found that the nuclear surface area, cytoplasma to nucleus and the parenchyma to interstitium ratios were lower in the cows fed 30 million IU vitamin D_3/d for 30 d than those in the control cows. Furthermore, cardiovascular calcification was observed within 10 d and became extensive 21 and 30 d after the start of vitamin D feeding. Dosages less than 30 million IU/d day were

not effective. The authors felt that feeding 30 million IU/d for 7 d prepartum might be effective and yet safe. This practice would be difficult to institute in the field due to the fact that calving date cannot be predicted accurately.

Magnesium Intake on Calcium Metabolism and Prevention of Parturient Paresis

Cows in dairy herds with a high incidence of PP often have subnormal blood Mg concentrations, particularly among the late pregnant cows (Sansom et al., 1983). In other species Mg deficiency was often accompanied by hypocalcemia. McManus and Heaton (1969) found that changes in plasma Mg concentrations produced by Mg deficiency in the rat varied with the dietary Ca content. When Ca intake was adequate, hypercalcemia occurred, but hypocalcemia occurred when Ca intake as well as Mg intake was restricted. Magnesium-deficient rats had low excretion of hydroxyproline, which was increased by the repletion of Mg. This suggested that Mg deficiency impaired bone resorption but might increase Ca absorption from the intestine and resorption in the kidney, because absorption of Mg and Ca from the gut and the renal tubules appears to occur by a common transport system (Alcock and MacIntyre, 1964). The effect of Mg deficiency on plasma Ca concentrations would depend on the balance of the above effects. These effects also may apply to dairy cows.

Calcium absorption diminishes progressively in all compartments of the bovine stomach with increasing Mg

concentrations in their contents. This inhibitory action of Mg on the ruminal absorption of Ca is evident not only at abnormally high Mg concentration (20 mg/100 ml) but also at physiological (10 mg/100 ml) and even at subnormal Mg concentrations (5 mg/100 ml) (Timet and Stojevic, 1982). Similar results also were observed in experiments with intestinal preparations from nonruminant animals (O'Donnell and Smith, 1973; Schatchter and Rosen, 1959) and intestinal preparations from sheep (Care and Van't Klooster, 1965).

An increase in Mg excretion by the kidney is accompanied by a marked rise in the excretion of Ca and vice versa in the dog, cat and rabbit (Mendel and Benedict, 1909a, 1909b). Recently Halse (1984) also found that renal conservation of Mg was increased during hypocalcemia postpartum or hypocalcemia induced by fasting in dairy cows.

Magnesium in the plasma exists in two forms, the ultrafiltrable and the nonfilterable. The ultrafiltrable Mg exists as free ions whereas the remainder is bound to albumin. The situation is similar to that of plasma Ca except a small portion of plasma Ca is in an ultrafiltrable but non-ionic form. In vitro experiments suggest that ionized Mg and bound Mg are interrelated by a simple dissociation equilibrium, which is governed by the law of mass action. Magnesium and Ca compete for the binding sites on the protein molecules (Car, 1955). The relative concentrations of total Ca and total Mg in the plasma have an important influence on the concentration of

ionized Ca and Mg. It is the concentrations of the ionized Ca and Mg that determine the excretion rate of each by the kidney. When excessive Ca was provided orally or parentally, it bound more protein sites, rendering more Mg free for excretion into the urine. When excessive Mg was provided, more proteins would bind Mg and release Ca and increase urinary excretion of Ca. Accordingly, hypocalcemia would leave more binding sites open to bind Mg. With Mg mobilized from the soft tissues and bone, the total plasma Mg may be increased without significantly increasing the concentration of ionized Mg in plasma. This may explain why hypermagnesemia is often associated with hypocalcemia, although the relative proportion of ionized and bound Mg in this case has not been determined. Any effect of this redistribution of Mg could result from the depletion of Mg from tissues, especially the bone, if the increased Mg in the plasma is mainly in the bound form.

About 70% of the body Mg is found in bone. In young rats and calves fed Mg deficient diets for extended periods, there was a 30% decline in bone Mg content (Watchorn and McCance, 1937; Wilson, 1964). Whether the same change would occur in adult cows needs to be verified.

The development of hypocalcemia in Mg-deficient animals appeared to be caused by a decrease in bone mobilization (Reddy et al., 1973; Welsh et al., 1981; Larvor and Labot, 1978). In growing chicks fed a Mg-deficient diet, a low Mg

content in the bone was accompanied by an increased weight and Ca content of the bone (Welsh et al. 1981), indicating the bone resorption process was inhibited.

In in vitro studies, Mg increased the release of Ca from aged rat bone by physicochemical processes involving exchange of Mg for Ca on the bone surface with no effect on phosphorus release. Magnesium also seemed to stimulate the normal bone resorption process involving breaking down of both the matrix and the mineral phases (McManus and Heaton, 1970; Raisz and Niemann, 1968). Bones from Mg-deficient calves were not responsive to PTH and 1,25-$(OH)_2D_3$ (Rayssiguir et al., 1977). Thus, Mg deficiency could be one of the factors that reduce the responsiveness of target organs to PTH and 1,25-$(OH)_2D_3$.

Parathyroid hormone stimulates bone resorption through the intermediate action of cyclic adenosine monophosphate (cAMP) (Rude, 1985). Adenylate cyclase requires Mg for cAMP generation as a component of the substrate and as an obligatory activator of the enzyme's activity. Intracellular Mg depletion in bone cells may result in impaired cAMP generation and a decreased biological effect in response to PTH (Rude, 1985). Skeletal adenylate cyclase was responsive to PTH 1-34, and this response was dependent on guanosine triphosphate (GTP) and Mg. Calcium causes a competitive inhibition of Mg-activated skeletal adenylate cyclase. Thus, it was suggested that a fall in intracellular Mg could render adenylate cyclase more susceptible to the inhibition of

prevailing intracellular Ca. Therefore, cAMP-mediated PTH induction of bone resorption would be impaired leading to hypocalcemia (Rude, 1985). In human patients with hypocalcemia and hypomagnesemia, parathyroid extracts had no effect on plasma Ca concentration, whereas intravenous infusion of $MgSO_4$ for 3 d restored the response to the parathyroid extracts and brought the plasma Ca concentrations back to normal (Estep et al., 1968, 1969). In the tibia of dogs fed Mg deficient diets, the basal cAMP production was very low and increased very little in response to PTH 1-34 infusion (Freitag et al., 1979)

Inadequate Mg in bone may impair bone resorption, resulting in hypocalcemia when the demand for Ca is increased suddenly, such as at the onset of lactation. Sansom et al. (1983) showed that supplementation of 25 g Mg/cow/d in the drinking water did not reduce incidence of milk fever in 8 herds with a history of about 10% incidence of milk fever, but this result was inconclusive, because no effort was made to balance the experimental animals according to susceptibility for PP. In two nonpregnant, nonlactating cows fed 5 g Ca/d, decreasing their Mg intake from 4 to 0.4 g/d depressed the exchangeable Ca pool and the rate of removal of Ca from and deposition into bone (Kronfeld, 1971). The cows probably were dependent on bone resorption to maintain Ca homeostasis due to the limited supply of Ca from the diet (5 g Ca /d). In lactating ewes a low Mg intake resulted in hypocalcemia and

hypomagnesemia. The plasma Ca as well as the Mg concentrations were restored when Mg was supplemented in the diet (L'Estrange and Axford, 1964). These results all indicate that adequate Mg supply is necessary for an optimal response of the bone to PTH stimulation.

Green et al. (1981) did not find significant differences in plasma concentrations of Ca, Mg, P and 1,25-$(OH)_2D_3$ between cows fed 6 g or 22 g Mg/d prepartum for about 14 days. A relatively high rate of Mg intake was provided for all cows 10 to 14 d before the experiment started. This might have repleted the Mg storage if it exists. Then, the effect of a relative Mg deficiency would be evident after the stored Mg was depleted, which might not have occurred within the short time period of the experiment. It seems likely that a high rate of Mg intake prepartum may reduce the absorption of excessive Ca, thus, stimulating secretion of PTH and also repleting bone Mg to ensure an adequate response of bone to PTH.

Van de Braak et al. (1987) showed that cows fed 71 g Mg/d had higher bone mobilization rate than cows fed 17 g Mg per day during the dry period. This agrees with the findings of Contreras et al. (1982), who found that hypomagnesemic cows were less able to mobilize Ca in response to hypocalcemia induced by intravenous infusion of EDTA solution. This indicates that adequate supply of Mg is important for the prevention of milk fever.

Availability of Mg may be reduced by the presence of high Ca concentration in the diet (Sansom et al., 1983). Verdaris and Evans (1976) also found that apparent Mg absorption was 38% with Ca intake of 24g/d, but was reduced to only 23% by Ca intake of 152 g/d. Part of the effects of restricted Ca intake prepartum observed by (Boda and Cole (1954) may be due to increased availability of Mg. Previous studies have involved relatively low dietary Ca intake when the effects of Mg intake was examined. Additionally, Mg excretion is increased by acidosis (Stacy and Wilson, 1970). Whether increasing Mg intake above requirement in cows fed acidodic diets with high Ca content would provide any advantage in the prevention of PP is not known.

Protein Intake on Calcium Metabolism and Prevention of Parturient Paresis

Dietary protein intake affected Ca metabolism of humans and some other species. Sherman (1920) first reported that addition of meat to a diet containing 390 mg of Ca caused an increase in urinary Ca without a concomitant increase in apparent Ca absorption in humans. Calcium balance was changed from positive to negative. He attributed these effects of meat to its high protein content. This was followed by numerous studies both in human beings and laboratory animals.

Margen et al. (1974) showed in a series of experiments that urinary Ca excretion was increased by an increase in

protein intake ranging from 6 to 600 g with Ca intake ranging from 100 to 2300 mg/d. Linkswiler et al. (1974) studied the effect of 48, 95 and 142 g protein intake on urinary Ca and Ca retention at Ca intakes of 500, 800 and 1400 mg/d. Urinary Ca increased with increasing protein intake but the effect on Ca absorption depended on Ca concentrations of the diet. At the 500 mg of Ca intake, Ca absorption was unaffected; but raising protein intake did enhance Ca absorption at the two higher rates of Ca intake. Calcium balance was reduced by high protein intake, indicating that Ca absorption was not increased enough to compensate the urinary excretion of Ca. When 48 g of protein/d were consumed, Ca retention was similar with 500, 800 or 1400 mg Ca/d. In contrast, protein intakes of 142 g caused negative Ca retention at all Ca intakes.

A change in protein intake affected urinary Ca very rapidly in man, although a few days were needed for the change to be completed. Allen et al. (1979) reported that eating a high protein diet significantly increased urinary Ca within 2 to 4 h. Several others suggested marked changes in urinary Ca excretion occurs within 24 h after a change in protein intake (Linkswiler et al., 1974; Margen et al., 1974). A slightly longer time seems to be required for urinary Ca excretion to stabilize when the protein intake was reduced compared with when the protein intake was increased. The protein effect on urinary Ca excretion also was sustained for long periods of time. Johnson et al (1970) reported that in young men,

urinary Ca was increased continuously with a high protein intake for as long as 45 d. Another study with young women showed the effect of protein intake on urinary Ca excretion persisted for 60 d (Hegsted et al., 1981).

It appeared that the most significant effect of dietary protein intake on Ca metabolism was to increase urinary Ca excretion. A relatively higher protein intake may increase Ca absorption slightly. However, any increase in Ca absorption associated with the addition of more protein to the diet was not sufficient to offset the increased loss of Ca in the urine (Yuen et al., 1984). Therefore, high protein intake lead to negative Ca balance.

Dietary protein intake seems to affect urinary Ca excretion by influencing renal functions either directly or indirectly. Several investigators have reported that glomerular filtration rate (GFR) was increased and fractional tubular reabsorption of Ca (FTRca) was decreased when protein intake was increased (Kim et al., 1979; Zemel et al., 1979; Schuette et al., 1980). The GFR was increased by 10 to 15% when protein intake was increased two to three folds. This accounts for about 10 to 15% of the elevation in urinary Ca that resulted from increases in protein intake (Linkswiler et al., 1981). In both men and women, FTRca was 98.5% when protein intake was 47 g. It decreased by about 1% with protein intake between 112 to 142 g/d. The decrease in FTRca

seems to account for most of the increase in urinary Ca caused by high protein intake (Linkswiler et al., 1981).

There is convincing evidence that the hypercalciuria caused by high protein intake partly is due to catabolism of excess dietary sulfur-containing amino acids (SAA) to sulfate and the subsequent excretion of sulfate in the urine. Lemann and Relman (1979) found that large supplement of methionine to men drastically increased urinary Ca excretion. The degree of hypercalciuria in rats produced by high protein feeding was proportional to the sulfur amino acid content of a number of different proteins and rates of protein intakes (Whiting and Draper, 1980). Increase in urinary Ca excretion was correlated positively with the increase in urinary sulfate and total renal acid excretion in old men and women when dietary protein was increased (Shuette et al., 1980). Wolgang et al. (1985) also found that the correlation between urinary sulfate and Ca excretions of 42 humans were marked ($r = .75$), and highly significant ($P < .01$), but only a modest correlation was found between urea and Ca excretion ($r = .33$). These results suggest that sulfate is a more important factor than urea in increasing Ca excretion.

Block (1984) have shown that a mixture of $CaCl_2$, $MgSO_4$ and $Al_2(SO_4)_3$ increased plasma hydroxyproline, indicating increased bone resorption. But it is not clear whether sulfate from the salt mixture had any specific role. Goodrich and Tillman (1966) showed in sheep that Ca retention was reduced by

sulfate supplementation as compared with elemental sulfur. Sodium sulfate is more bioavailable than elemental sulfur (Goodrich and Tillman, 1966). It can be expected that more sulfur was absorbed in sheep fed Na_2SO_4 than those fed elemental sulfur. Theoretically, Na_2SO_4 should not cause acidosis as sulfur amino acids would. Thus, the decrease in Ca retention caused by Na_2SO_4 might be independent of the acidotic effect. Shuette et al. (1980) suggested that increased sulfate concentration in the renal tubules may decrease Ca reabsorption by increasing the electronegativity of the lumen, because sulfate ion is reabsorbed poorly. An increased concentration of sulfate also may decrease Ca reabsorption by forming complexes with Ca. Increased excretion of Ca may also activate the Ca homeostatic mechanisms to increase Ca mobilization in the body.

Zemel et al. (1981) compared the effect of increasing dietary protein intake from 50 to 150 g with those of increasing only dietary intake of SAA. Addition of SAA to 50 g protein diet to simulate the amount of SAA in the high protein diet caused an increase in urinary Ca excretion and a decrease in fractional tubular reabsorption rate of Ca, but these changes were only 43 and 44% of those caused by the high protein intake. Renal acid excretion, however, was affected to the same extent by the increase in SAA as by the increase in protein intake. It appeared that part of the effect of protein on urinary Ca were due to factors other than sulfate.

Whether high protein intake affects the activities of Ca regulatory hormones is not understood fully. The fasting plasma concentration of PTH and 1,25-$(OH)_2D_3$ were not affected by dietary protein intake, suggesting their involvement was limited in affecting urinary Ca excretion in man (Allen et al., 1979; Kim and Linkswiler, 1979; Schuette et al, 1980, 1981).

High protein intake may affect activities of some other hormones, such as insulin, and indirectly affect Ca mobilization from bones. Allen et al. (1981) suggested that enhancement of insulin production by an influx of amino acids might be a cause of the hypercalciuretic effect of high protein diets. They found that serum insulin concentrations were higher for humans fed a meal with 45 g protein than for those fed a meal with 15 g, although no differences were found in fasting concentrations of serum insulin between the two groups (Allen et al., 1979). In contrast, It was demonstrated that consumption of a high protein diet was calciuretic even in alloxan-diabetic rats, suggesting that insulin may not be necessary for the hypercalciuretic effect to occur with high protein diets (Whiting and Drape, 1981).

A recent study with growing horses showed that, compared to a diet with a low protein content (9% CP, DM basis), diets with 14 or 20% CP resulted in higher rate of bone accretion and higher rate of bone resorption, suggesting that a high protein intake may increase bone turnover rates (Schryver et

al. 1987). However, the effect became insignificant at 12 mo of age. Only three horses from each group were used to estimate the rates of bone accretion and bone resorption. The results were inconclusive.

The number of studies on the effect of protein intake and sulfur intake on Ca metabolism of dairy cows is limited. Curtis et al. (1984) used epidemiological methods to show that protein intake during the dry period was an important factor, just second to the effect of parity and previous milk yield on the incidence of PP in dairy cows, indicating that protein intake may affect Ca metabolism in dairy cows. Whether manipulation of dietary protein intake has any value in PP prevention will depend on more specific information about the effects of protein intake on Ca metabolism.

Dietary Cation-Anion Difference on Acid-Base Status, Calcium Metabolism and Prevention of Parturient Paresis

The concept of dietary cation-anion difference (DCAD) relates to the difference between total fixed (non-metabolizable, bioavailable) cations and total fixed anions in the diet. It is usually calculated as (Na + K) - (Cl + S) in meq/kg diet DM (Dishington, 1975; Block, 1984). Because Na, K and Cl dissolve nearly completely and are absorbed nearly completely and have a larger impact than other minerals, DCAD also has been calculated simply as Na + K - Cl (Mongin, 1981). The DCAD has been suggested to affect acid base balance and Ca

metabolism in other species and cows (Hurwitz et al., 1973; Mongin, 1981; Block, 1984; Fredeen et al., 1988a, b; Oetzel et al., 1988).

Effect on Acid-Base Balance

Acid-base balance depends on the concentration of hydrogen ions (H^+) in the body. Any changes in the concentration of H^+ are counteracted by the buffering systems, respiratory and renal compensation (Kilderberg, 1968). Buffers minimize pH changes by converting stronger acids or bases into weaker acids or bases and their salts. The plasma buffers are proteins, phosphates and carbonates, whereas in the red blood cells, hemoglobin and oxyhemoglobin, phosphates and carbonates act as buffers (Klahr, 1982). By virtue of its unique properties, the bicarbonate system contributes half of the whole blood buffering capacity (Chan, 1981). The Henderson and Hasselbalch equation defines blood pH as

$$pH = pK + \log[(HCO_3)/(.03 \times pCO_2)]$$

where the pK is the dissociation constant of carbonic acid, HCO_3 is the bicarbonate concentration, .03 is the solution constant of carbonic acid and pCO_2 is the partial pressure of CO_2 in the blood. This shows that any factors that directly or indirectly change the concentration of HCO_3, PCO_2 or their ratio in the blood would affect acid-base balance. Acidosis or alkalosis caused by changes in pCO_2 are respiratory acidosis or alkalosis, whereas acidosis and

alkalosis caused mainly by changes in the concentration of HCO_3 are metabolic acidosis and alkalosis. Respiratory compensation of acid-base disturbances is through changes in the respiration rate with resulting changes in blood pCO_2. Metabolic compensation of acid-base disturbances is through changes in reabsorption and excretion of HCO_3 and H^+, and excretion of ammonia in the renal tubules (Chan, 1981). Under acidotic conditions, CO_2 and H_2O are converted to HCO_3 and H^+ through the action of renal carbonic anhydrase. While HCO_3 is reabsorbed, H^+ that passes on is buffered in the renal tubules by filtered monohydrogen phosphate to form dihydrogen monophosphate, which is quantifiable in the urine as titratable acidity (Chan, 1972). Glutamine is acted on by glutaminase to produce ammonia, which diffuse easily into the renal tubules to combine with H^+ to form ammonium (Chan, 1981).

Dietary cation-anion difference does not necessarily determine the acidogenic or alkalogenic properties of diets, but the participation of the fixed ions and non-fixed ions associated with them result in changes in acid-base balance (Mongin, 1981; Chan, 1981). When DCAD is 0, the number of fixed cation equivalents equals the number of fixed anion equivalents. A good example is NaCl, which contains equal number of positive Na ion and negative Cl ion. Assuming complete absorption, the consumption of NaCl would not affect acid-base status of the body. When DCAD is positive, more

fixed cation equivalents than fixed anion equivalents exist in the diet. This means that some fixed cations, such as Na ions are balanced by anions that are not fixed or anions that can be further metabolized in the body to other forms. For example, citrate can be metabolized to form CO_2 and H_2O with a negative HCO_3 generated to balance the positive Na ion. When DCAD is negative, more fixed anions than fixed cations exist in the diet. Some dietary anions, such as Cl ions may be balanced by cations that can be further metabolized. For example, NH_4 can be used for urea synthesis with one H^+ left to balance Cl^-. These demonstrate that salts consisting of both fixed ions and non-fixed ions could affect acid-base balance, because the nonfixed ions are metabolized, resulting in generation of H^+ or HCO_3.

In addition, salts consisted of only fixed ions also could affect acid-base balance if there are differences between the absorption efficiency of cations and that of anions. Both Ca ions and Cl ions from $CaCl_2$ are fixed ions. Only a portion of Ca is absorbed, but Cl is absorbed almost completely in ruminants (NRC, 1989). The unabsorbed Ca precipitates with HCO_3 with release of H^+, which decrease pH in the gut, and in the plasma when absorbed.

The effect of DCAD on acid-base status has been characterized in nonruminant animals (Hurwitz et al., 1974; Mongin, 1981). Acid-base balance is normal when (Na + K) - Cl is 250 meq/kg DM. Greater excess of cations causes metabolic

alkalosis, whereas excessive anions cause metabolic acidosis. Acid-base status of goats was normal when DCAD (Na + K - Cl) was 40 to 50 meq/kg DM (Fredeen et al., 1988a). Metabolic alkalosis was induced when DCAD was >850 meq/kg. Metabolic acidosis was induced when DCAD was <100 meq/kg DM. The authors based these conclusions on results from blood gas analysis, determination of urinary titratable acidity and ammonium excretion. That negative DCAD caused metabolic acidosis has been shown also in sheep and dairy cows (Vagg and Payne, 1970; Kendall et al., 1969; Verdaris and Evans, 1974).

Effects on Calcium Metabolism and Prevention of Parturient Paresis

The relationship between DCAD and Ca metabolism and PP was first suggested by researchers in Norway. They found that PP was induced by diets with beets, but prevented by diets with silage preserved with mineral acids (HCl and H_2SO_4), regardless of Ca and P intake (Ender, 1962). Analysis of the feed components showed that the beets were very high in Na and K content but the silage were high in Cl and S, in addition to the pH differences between the diets. The opposite effects of the two types of diets were verified by feeding experiments with ordinary diets supplemented with either Na_2CO_3 + $NaHCO_3$ or $CaCl_2$, $Al_2(SO_4)_2$ plus $MgSO_4$, to match the concentrations of Na, K, Cl and S in beets and silages used previously (Dishington, 1975). It was found that 13 of the 14 cows fed Na_2CO_3 + $NaHCO_3$ suffered from PP, whereas 12 of the 13 cows fed $CaCl_2$,

$Al_2(SO_4)_2$ plus $MgSO_4$ were healthy with only one other cow showing borderline symptoms of PP. Reducing the dietary pH alone did not reduce incidence of PP, but addition of a mixture of $CaCl_2$, $Al_2(SO_4)_3$ and $MgSO_4$ reduced PP incidence significantly. This was confirmed again by Dishington and Bjornstad (1982), who showed that 17 out of 20 cows did not succumb to PP when the DCAD was -943 meq/kg with a concentrate pellets supplemented with $CaCl_2$, $Al_2(SO_4)_3$ and $MgSO_4$.

Studies with five PP prone cows over six yr showed that cows had PP only in the years they were fed diets with positive DCAD, but cows with a history of PP did not have PP in the years they were fed diets with negative DCAD (Dishington, 1975). These results contradict the common believe that cows with history of PP occurrence would very likely be affected repeatedly, but further emphasized the preventive effect of negative DCAD on PP. However, these results were from studies with relatively small number of cows under Norwegian conditions.

It was not until 1984 that this idea was applied to conditions in North America. Block et al. (1984) reduced DCAD from 330 meq/kg DM in the control diet to -128 meq/kg DM in the treatment group by supplementing the diet with a mixture of $CaCl_2$ (.23% diet DM), $Al_2(SO_4)_3$ (.86%) and $MgSO_4$ (.74%). Incidence of PP was 47% for the control group compared with 0% for the group fed the diet with negative DCAD during the

experiment. Whether any of the minerals added with the mixture had any specific effect was not clear.

Ammonium chloride also induced acidosis and increased bone resorption in goats and cows (Vagg and Payne, 1970). In a German study cited by Jonsson (1978), feeding 110 to 140 g/d for 21 d prepartum reduced the incidence of PP. However, the use of NH_4Cl was considered unsuitable for PP prevention, because it is unpalatable and potentially toxic when added to conventional diet (Jorgensen, 1974). Oetzel et al. (1988) added a mixture of NH_4Cl and $(NN_4)_2SO_4$ (100 g each/cow/d) to reduce DCAD from 189 meq/kg to -75 meq/kg DM during the last 21 d of gestation. Incidence of PP was lowered from 17% to 4%, and the risk of hypocalcemia (ionized Ca < 4 mg/dl) was reduced by 10-fold by the diet with negative DCAD.

Dietary cation-anion difference probably affects Ca metabolism directly or indirectly by affecting acid-base balance. It is still controversial whether DCAD alters absorption of Ca from the intestine. Ender and Dishington (1970) showed that high positive DCAD reduced apparent Ca absorption and caused negative Ca balance, whereas negative DCAD increased Ca absorption and lead to a positive Ca balance during the first few days after parturition. Lomba et al. (1978) summarized data from a number of experiments, and found that diets with negative DCAD resulted in higher apparent Ca absorption than diets with positive DCAD fed to cows with positive Ca balance. Verdaris and Evans (1976) also found

that an acidogenic diet (with H_3PO_4) increased Ca absorption when dietary Ca was high. It was shown in sheep with radioisotopic methods that diets with negative DCAD (due to supplemental NH_4Cl) increased both Ca absorption and endogenous excretion with only minor effect on the net Ca absorption (Braithwaite, 1972).

The DCAD may affect Ca regulatory hormones indirectly by affecting acid-base balance. In rats chronic metabolic acidosis depressed conversion of 25-$(OH)D_3$ to 1,25-$(OH)_2D_3$ (Lee et al., 1977). Bushinsky et al. (1985) also found that metabolic acidosis reduced blood concentrations 1,25-$(OH)_2D_3$. However, studies in dogs showed that the stimulative effect of PTH on 1,25-$(OH)_2D_3$ formation were enhanced by metabolic acidosis (Burnell, 1971). The responses may be different among species. Recently Gaynor et al. (1989) showed that plasma concentrations of 1,25-$(OH)_2D_3$ were related inversely to DCAD in dairy cows (The DCAD expressed as (Na + K) - Cl were 220, 599 and 1258 meq/kg DM). They suggested that acidosis might increase the responsiveness of renal tissues to PTH, but concentrations of PTH were not determined in the study. It also is possible that acidosis could have a direct effect on the renal hydroxylase responsible for the conversion of 25-$(OH)D_3$ to 1,25-$(OH)_2D_3$.

The DCAD also may have some effect on blood Ca concentrations due to its effect on acid-base balance. Calcium is present in the plasma in three major forms:

ionized, protein-bound and complexed (e.g. with citrate). The ionized form is the biologically active form, representing about 50% of the total plasma Ca (Ender et al. 1962). Moore (1970) examined in vitro the effect of blood pH on ionized Ca concentrations over a pH range of 6.8 to 7.8. He demonstrated instantaneous and completely reversible changes in ionized Ca concentration in response to pH change of human blood. Ionized Ca decreased in a linear or slightly sigmoid fashion with increasing pH. He suggested that the effect of pH on ionized Ca may be due to competition between Ca^{++} and H^{+} for binding sites on serum proteins. Plasma concentrations of total Ca were higher around parturition in dairy cows when DCAD was negative (Block, 1984). In goats plasma concentrations of total Ca was also higher when DCAD was negative (Fredeen et al., 1988a). Oetzel et al. (1988) showed that negative DCAD increased plasma concentrations of both ionized Ca and total Ca, but not the ratio of the two on the day of parturition. Because blood pH was not determined in the study, it is difficult to assess whether there was enough change in blood pH to affect the ratio (ionized Ca/total Ca).

The DCAD also could affect urinary excretion of Ca via changes in acid-base balance. Acidosis was shown to increase urinary excretion of Ca in man, dogs, sheep, goats and other species (Barzel, 1981). The hypercalciuretic effects of negative DCAD also have been observed in dairy cows (Horst and Jorgensen, 1973; Vagg and Payne, 1970) and in goats (Fredeen

et al., 1988b). Increased urinary Ca excretion reflects the difference between the rate of glomerular filtration and tubular reabsorption of Ca in the kidney. During chronic metabolic acidosis, glomerular filtration rate fell slightly, but Ca excretion increased markedly, indicating that increased Ca excretion was mainly due to the inhibition of Ca reabsorption caused by acidosis (Lemann et al., 1967; Barzel and Jowsey, 1969). Hypercalciuria also was observed in parathyroidectomized animals rendered acidotic and in hypothyroid patients given NH_4Cl (Barzel, 1981). It appears that PTH was not involved in the effect of acidosis on urinary Ca excretion.

Acidosis caused hypercalciuria without increasing Ca absorption, resulting in negative Ca balance (Lemann et al., 1966). Bone mineral content decreased after administration of acid to rats (Barzel and Jowsey, 1969). This indirectly suggests that Ca mobilization from bone may be enhanced by acidosis.

Bone maintains some amorphous Ca phosphate and Ca carbonate located on the less labile hydroxyapatite crystal surfaces (Termine and Posner, 1966). These mineral salts can be solubilized in the presence of an increased concentration H^+ with the release of buffer bases, such as phosphate and carbonate) (Barzel, 1969; Bushinsky and Lechleider, 1987). It was estimated that adult human skeleton has 35,000 meq of potentially available bases (Goodman et al. 1965). It is this

large store of bases that is believed to be drawn upon to maintain nearly normal plasma bicarbonate concentrations for months in patients with chronic renal acidosis. Calcium released during the process can increase Ca input to the exchangeable Ca pool.

Hydrogen ion may play an important role in the resorption of bone minerals (Vaes, 1968). Decreasing pH increases the solubility of bone mineral, allowing access of enzymes, such as collagenase, lysosomal enzymes including acdiphosphatase, glucuronidase, galactocidase to demineralized bone matrix (Mundy and Raisz, 1981). There appeared to be an acidic pH in the zone between the resorbing osteoclast and the bone mineral, indicating that proton may be generated by osteoclasts to dissolve bone mineral (Mundy and Raisz, 1981). Carbonic anhydrase facilitates osteoclastic bone resorption by generating H^+ from CO_2 by the CO_2 hydration reaction (Hall and Kenny, 1987). Inhibition of carbonic anhydrase inhibited Ca release from bone in vitro (Bushinsky et al., 1985). Enzymes involved in bone resorption, such as succinate dehydrogenase, acid phosphatase, may be dependent on H^+ for optimal activity (Mundy and Raisz, 1981). Beck and Webster (1976) showed that in parathyroidectomized rats, bone resorption was increased by NH_4Cl in the diet, suggesting that acidosis may stimulate osteoclasts, independent of PTH.

A recent study by Fredeen et al. (1988b) showed diet with negative DCAD increased bone resorption rate measured by the

^{45}Ca kinetics techniques, confirming the results of earlier work by Vagg and Payne (1970). It appears that DCAD affects all aspects of Ca metabolism indirectly by affecting acid-base status. Negative DCAD induces metabolic acidosis and increases the size or the turnover rate of exchangeable Ca pool. Therefore, animals fed a diets with negative DCAD potentially can better maintain blood Ca concentrations when the demand for Ca is increased suddenly.

Better effect was observed when the diet with negative DCAD was also high in Ca content (Dishington, 1975). Ender and Dishington (1970) showed that cows fed diets with negative DCAD and high Ca intake maintained higher Ca balance at parturition. Oetzel et al. (1988) also observed that cows fed diets with negative DCAD and high Ca intake maintained a higher plasma Ca concentration at parturition. These results support the suggestion that Keeping DCAD negative and dietary Ca relatively high are important for PP prevention.

Summary and Hypothesis

The blood Ca concentration is regulated strictly within a physiologically normal range in dairy cows as in other species. Maintenance of plasma Ca concentrations is dependent on the rates of Ca inflow to and outflow from the exchangeable Ca pool. The most limiting factors are the efficiency of Ca absorption from the gut and the rate of Ca mobilization from bone. Restricting Ca intake prepartum increased Ca

mobilization from bones and reduced incidence of PP when the intake was restricted to less than 20 g/d. However, Less dramatic reduction in Ca intake were not successful in preventing PP. Sometimes such dramatic reduction in Ca intake is not possible or not economical. Feeding large doses of vitamin D or injection of vitamin D metabolites appeared to be effective under certain conditions, but the use of them are restricted by the fact that exact calving date is not predicted accurately. Long term feeding of large amounts of vitamin D or repeated injection of vitamin D metabolites may lead to toxicity. More effective and practical measures are needed for the prevention of PP.

Increased protein intake appeared to increase Ca mobilization in other species. Epidemiological data suggest that higher protein intake during the dry period was related to lower incidence of PP. However, Information on the effect of dietary protein intake on Ca metabolism in dairy cows is lacking for evaluating the potential value of increased protein intake prepartum in the prevention of PP.

Manipulation of DCAD affected acid-base balance and Ca metabolism in various species. Diets with negative DCAD and high concentration of Ca were suggested for the prevention of PP. Feedstuffs for dairy cows usually have positive DCAD. Acidogenic salts are needed to make DCAD negative. Mixtures of NH_4Cl and $(NH_4)_2SO_4$ were shown to reduce incidence of hypocalcemia. Further studies are needed to provide

information on other metabolic effects of these mixtures. Magnesium plays an important role in Ca metabolism. Very low Mg intake resulted in hypomagnesemia and low mobilization of Ca from bone. Magnesium absorption was reduced by high Ca intake. Urinary excretion of Mg was increased by acidosis. It is not clear whether Mg intake needs to be increased for cows fed diets with negative DCAD and high Ca concentrations may be benefical. Additionally, PP and subclinical hypocalcemia may increase risks of other metabolic disorders, such as retained placenta, dystocia, ketosis, metritis, mastitis and displaced abomasum. Prevention of PP and hypocalcemia may reduce the incidence of the incidence of these disorders, resulting in improved reproductive and productive performance of dairy cows. The effects of diets with negative DCAD on all these aspects needs to be validated on a large numbers of cows.

The specific objectives of the proposed studies were: 1) to determine the effects of dietary protein intake on acid-base status and Ca metabolism in nonlactating nonpregnant Jersey cows; 2) to further study the effects of mixtures of NH_4Cl and $(NH_4)_2SO_4$ on acid-base status and Ca metabolism in nonlactating and nonpregnant Jersey cows; 3) to study the effects of Mg intake on acid-base status and Ca metabolism of nonpregnant and nonlactating Holstein cows all fed diet with negative DCAD; and, 4) to test the effectiveness of diets with negative DCAD on Ca metabolism, incidence of PP and other

disorders, and production performance in a large commercial dairy herd.

CHAPTER 3
EFFECTS OF SUPPLEMENTAL PROTEIN ON ACID-BASE BALANCE AND CALCIUM METABOLISM OF NONLACTATING JERSEY COWS

Introduction

Dietary protein intake affects Ca metabolism of humans and other species (Yuen et al., 1984). Increased protein intake by humans increased urinary Ca excretion, resulting in negative Ca balance (Linkswiler et al., 1974; Margen et al., 1974). Excessive dietary sulfur-containing amino acids (SAA) caused acidosis in humans (Lemann and Relman, 1979). The degree of hypercalcemia induced by high protein feeding in rats was proportional to SAA content of several protein sources (Whiting and Drape, 1981). When dietary protein content was increased, urinary excretion of sulfate, but not urea, was correlated highly with urinary Ca excretion (Wolfgang and Ritz, 1985). This suggested that SAA may be responsible for effects of dietary protein intake on Ca metabolism. However, other factors also may be involved because supplementing SAA in a low protein diet (50 g/d) to amounts present in a high protein diet (150 g/d) increased urinary Ca excretion by men only to 43% of that induced by the high protein diet (Zemel et al., 1981). Increased amounts of amino acids from protein digestion might increase Ca

absorption or cause some hormonal changes leading to increased bone resorption (Allen et al., 1981).

Information on effects of dietary CP or S supplementation on Ca metabolism of dairy cattle is limited. Milk fever is characterized by inability of dairy cows to mobilize enough Ca (via bone resorption or absorption) to maintain blood Ca concentrations at and around parturition when demand for Ca is increased suddenly due to initiation of lactation (Rambaerg et al., 1975). In a study in New York, epidemiological data showed that increased protein intake during the dry period might reduce incidence of PP of dairy cows (Curtis et al. 1984). This suggested that Ca metabolism may be affected by protein intake in dairy cows, as observed in other species. The objective of this experiment was to study the effects of supplemental dietary protein on acid-base status, Ca balance, and responses to hypocalcemia induced by intravenous infusion of Na_2EDTA solution.

Materials and Methods

Six nonlactating, nonpregnant multiparous Jersey cows (average 6.7 yr old) were assigned randomly to treatment sequences in two 3 X 3 Latin squares balanced for carryover effects. Cows were blocked to squares according to initial BW. Cows in square 1 averaged 408 kg and cows in square 2 averaged 493 kg BW at the beginning of the experiment. Three treatment diets had 11%, 15%, or 19% CP contents, DM basis.

Table 3-1. Formulation of diets with varying supplemental CP concentrations.

Ingredient	Dietary CP content, % DM		
	11	15	19
	----------(% DM)----------		
Cottonseed hull	60.00	60.00	60.00
Soybean meal, 48% CP	10.80	10.80	10.80
Ground corn	27.94	18.98	10.03
Hydrolyzed feather meal	...	3.87	7.74
Distillers dried grain with solubles	...	5.35	10.70
Dicalcium phosphate	.280	.140	.131
Trace mineral NaCl[1]	.100	.100	.100
Calcium carbonate	.500	.485	.470
Magnesium oxide	.012	.016	.020
Sodium chloride	.180	.162	.140
Sodium bicarbonate	.190	.095	...

[1]Composed of 95 to 99% NaCl, .35% Zn, .34% Fe, .20% Mn, .033% Cu, .007% I, and .005% Co.

The 11% CP diet consisted of cottonseed hulls, ground corn and soybean meal (Table 3-1). For diets with higher protein contents, hydrolyzed feather meal and dried distillers grains with solubles each supplied 50% of the supplemental protein by replacing equal amounts of ground corn. All diets were formulated to meet or exceed nutrient requirements for dry pregnant cows recommended by NRC (1989) except for CP and S. Concentrate mixes were prepared before the experiment commenced in amounts required for the entire experiment.

All six cows were kept in individual tie stalls in a flat barn equipped with evaporative cooling. Cows had free access to drinking water. They were allowed to exercise in an open lot twice weekly for 4 h each day except during collection periods. They were fed the 11% CP diet throughout the adjustment period (14 d). Thereafter, they were fed individually assigned experimental rations in two equal portions at 0700 and 1800 h daily. Amount of ration offered was restricted to near maintenance energy requirements and kept the same throughout the experiment. Concentrates and cottonseed hulls were weighed separately and mixed manually at the time of feeding.

Samples of concentrates and cottonseed hulls were taken weekly and pooled within period. Daily water intake was measured by in-line flow meters. Cows were weighed on a digital electronic scale at the beginning and end of each period. On d 14, urethral catheters were placed in all cows.

From d 15 through 21, total excretion of urine was collected into plastic containers kept in ice, and feces were collected in a trough placed behind each cow. Amounts of urine and feces excreted were weighed daily. Weights of urine were converted to volume using an average specific gravity (1.03). Daily, 1% of urine volume was taken for analyses of concentrations of creatinine and ammonium, pH and titratable bases. One part of concentrated HCl:50 parts of urine was added to the remainder of the urine, and mixed well and another 1% was taken for mineral analyses. Daily samples were pooled within cow-period, and stored (-10^{0}C) in plastic bottles until analyzed. Daily fecal excretion was mixed thoroughly and 5% (by weight) sampled and stored frozen in plastic bags. At the end of the experiment, fecal samples were thawed, subsampled and pooled within cow-period.

On 2 d of each collection period, blood samples were taken anaerobically from the jugular vein before the 0700 h feeding for blood gas analysis. Another 25 ml of blood were taken into two 14-ml plastic tubes coated with ammonium heparin (20 U/ml). After centrifugation at 2800 x g for 20 min, plasma was harvested and stored in small plastic tubes until analyzed for creatinine and minerals. After total collection was completed, on the afternoon of d 22 of each period, catheters (Abbocath-T 14G X 5 1/2", radiopaque FEP teflon I. V. catheter, Abbott Hospitals, Inc., North Chicago, IL) were inserted into both jugular veins. Physiological

saline with 150 U ammonium heparin/ml was kept in the catheters. A preliminary experiment was performed several weeks before the main experiment to establish the EDTA infusion rate needed to cause signs of severe hypocalcemia within 2 to 3 h of initiation of infusions. The EDTA infusion did not cause significant changes in plasma Mg. This finding was supported by studies of Ramberg et al. (1967). Therefore, in the main experiment a 10% EDTA saline solution (pH adjusted to 7.4 with NaOH) was infused simultaneously into three cows (square 1 on d 23 and square 2 on d 24) at an average rate of 3.14 ml/min (.314 mg/min or .041 mg/h/kg BW). Infusion of EDTA was terminated for all three cows within a square when one of the 3 cows showed signs of hypocalcemia such as excessive salivation, teeth-grinding, trembling and inability to stand. A 25-ml blood sample was taken from the jugular vein opposite the side used for EDTA infusion at the beginning of infusion and at 30-min intervals for 15 h after the beginning of EDTA infusion. The cows were not fed until the last blood sample was taken. Samples were taken into 30-ml syringes and then transferred to two 14-ml plastic tubes coated with ammonium heparin (20 U/ml). After centrifugation (2800 x g for 20 min), plasma was harvested and stored in (-10°C) small plastic tubes until analyzed.

Fecal subsamples were dried for chemical analysis at 60^0C for 72 h and at 100^0C for 24 h for DM analysis. Feed and dried fecal samples were ground through 2-mm screen (Thomas-

Wiley Mill, Model 4, Thomas Scientific, Philadelphia, PA). Feed DM and CP (macroKjeldahl) concentrations were determined according to AOAC (1970). Samples of feed and feces were ashed at 550°C overnight. After dissolving the ash in 3 N HCl, Ca, Mg, Na, and K were determined by atomic absorption spectrophotometry (Model 5000, Perkin Elmer, Inc., Norwalk, CT). Phosphorus was determined by colorimetry (Fiske and Subbarow, 1925). For S determination, samples were digested in 50-ml beakers with 99% (wt/vol) magnesium nitrate for 1 h or until reaction ceased on a hot plate at 180°C, and then ashed in a muffle furnace at 500°C for 4 h. Once the ash was dissolved in 2.75 N HCl, S was determined by turbidimetry with polyethylene glycol 6000 as a suspending reagent (Lunquist et al., 1980; Ma and Chan, 1973). Blood pH, bicarbonate, and total CO_2 were determined using a blood gas analyzer (Model 1304, Instrumentation Lab, Lexington, MA). Plasma EDTA-free Ca was determined by colorimetry (Gindler and King, 1972). For plasma and urine samples, the same methods as for feed and fecal samples were used to determine concentrations of total Ca, Mg, P, and S after samples were deproteinized with 10% TCA. Concentrations of plasma and urine creatinine were determined according to Henry et al. (1974).

Urine pH was determined daily on unacidified sub-samples. Titratable base and ammonium concentrations were determined by titration of nonacidified urine samples (Chan, 1972; Lin and Chan, 1973). Net base excretion was calculated as (titratable

base - ammonium) X urine volume per day. Concentrations of mineral and creatinine in plasma and urine, and urine volume collected on the same days, were used to calculate fractional renal excretion ([(urine mineral concentration/plasma mineral concentration) X (plasma creatinine concentration/urine creatinine concentration)] X 100) (Lane and Merritt, 1983).

Statistical analyses were performed by the method of least squares analysis of variance, using general linear model procedures of SAS (Barr et al., 1982). Independent variables in the mathematical model were square, cow, period, and treatment. Sampling day was included in the model for plasma mineral concentrations and fractional excretions. Orthogonal contrasts were made to examine the linear and quadratic effects of supplemental dietary protein on dependent variables. The quadratic effects were not significant for any dependent variables. Thus, the probabilities presented in tables, as well as text, are all for linear effects of protein supplementation. Tests for heterogeneity of regression (Snedecor and Cochran, 1980) were used to examine the effects of treatments on changes in plasma total Ca and EDTA-free Ca over time after start of EDTA infusion.

Results

One cow fed the 15% CP diet developed severe anorexia midway through period 1 for unknown reasons and was replaced by another cow kept in reserve under similar conditions.

Table 3-2. Laboratory analysis of chemical composition of experimental diets with varying supplemental CP content.

Item	Dietary CP content, % DM		
	11	15	19
DM, %	87.5	88.2	89.0
NE_l, Mcal/kg[1]	1.21	1.17	1.12
CP, %	11.1	14.6	18.8
Ca, %	.40	.40	.43
P, %	.26	.28	.28
Mg, %	.16	.18	.19
K, meq/kg	302	302	320
Na, meq/kg	91	91	91
Cl, meq/kg	55	52	64
S, meq/kg	69	131	200
(Na+K)-(Cl+S), meq/kg	+269	+210	+147

[1]Calculated from values for individual feedstuffs listed by NRC (1989).

Thus, no data were collected for the replacement cow during period 1.

Chemical composition of the treatment diets is in Table 3-2. In addition to major differences in CP concentrations, S concentrations differed among treatments. Water intake averaged 22.3±.94 L/d (mean ± SEM). Feed DM intake was controlled at 1.68% of BW (averaging 7.13 ± .03 kg/d). Apparent DM digestibility was similar among treatments, averaging 57.6±.8%. Daily CP intakes were .79, 1.04, and 1.34 ± .01 kg/d for 11, 15, 19% CP treatments, respectively. Body weight increased slightly (4.9±5.7 kg) during each period.

Concentration of Ca, Mg, and P in plasma was not affected, but concentrations of S increased ($P < .06$) with increasing dietary protein supplementation (Table 3-3). Jugular blood pH and total CO_2 tended to decrease, and base excess decreased ($P < .07$) as supplemental protein increased (Table 3-4). Urine pH was not affected. Urinary ammonium excretion increased, but titratable base and net base excretion decreased with increased supplemental protein (Table 3-4).

Urinary Mg and S excretion increased as dietary protein supplementation increased (Table 3-5). Fecal excretion of Ca, P, and Mg were not affected by dietary treatments (Table 3-5). Fecal S excretion increased when dietary protein intake increased. Whole body balance of Ca, P, Mg, and S were not affected by supplemental protein intake (Table 3-5). Total

Table 3-3. Effect of dietary supplemental protein on blood plasma concentrations of macrominerals.

Plasma minerals	Dietary CP content, % DM 11	15	19	SEM	P[a]
	----------(mg/dl)----------				
Ca	9.84	9.84	9.68	.08	<.55
P	6.72	7.03	6.50	.25	<.56
Mg	2.09	2.19	2.14	.05	<.36
S	2.50	2.50	3.30	.19	<.06

[a]Probability for the linear effects of supplemental dietary CP content; no evidence of curvilinearity.

Table 3-4. Effect of supplemental dietary protein content on acid-base status of Jersey cows.

Item	Dietary CP content, % DM 11	15	19	SEM	P[a]
Blood					
pH	7.348	7.405	7.307	.01	<.18
BE[1], meq/L	1.11	1.15	.92	.23	<.03
HCO_3, mmol/L	26.92	26.47	25.55	.25	<.14
TCO_2, mmHg	28.43	27.79	27.12	.31	<.25
Urine					
pH	8.625	8.422	8.388	.09	<.19
NH_4, meq/d	40.68	56.29	80.51	1.60	<.01
TB[2], meq/d	846.7	813.9	725.5	21.98	<.02
NBE[3], meq/d	805.98	757.59	644.97	21.14	<.01

[a]Probability for linear effects of supplemental dietary CP content; no evidence of curvilinearity.

[1]Base excess.

[2]Titratable bases equals amount of HCl required to titrate urine to pH 7.4.

[3]Net base excretion equals titratable base minus ammonium.

urinary Ca and P excretion (Table 3-5) and fractional excretion of Ca, P, Mg, and S was not affected by supplemental dietary protein (Table 3-6).

Infusion of EDTA continued for about 150 min, varying slightly between periods and squares. Average total volume of 10% EDTA saline solution infused into each cow was 458 ml (equal to 45.8 g EDTA); the EDTA chelated approximately 4.9 g of Ca from plasma. The lowest average EDTA-free Ca concentration was 3.27±.43 mg/dl, with no differences among treatments.

Responses of plasma EDTA-free Ca concentrations over sampling time after the beginning of EDTA infusion were best estimated by a 5th degree polynomial (R^2 = .73) (Figure 3-1). A similar trend was observed for changes in the concentrations of total plasma Ca over sampling times (R^2 = .64). The data were examined first as one set, and then as several subsets. The declining phase of plasma EDTA-free Ca (from the beginning to end of infusion (about 2.5 h), the ascending phase (from end of infusion to 7 h after infusion ceased, when EDTA-free Ca concentrations approached near pre-infusion values), and the later part of the recovery phase (from 7 h to 12 h after infusion ceased) were all similar among treatments. Homogeneity of regression showed that the responses of plasma EDTA-free Ca, total Ca and EDTA-bound Ca over sampling time after the initiation of EDTA infusion were similar for all three treatment groups. Least squares means of plasma

Table 3-5. Effect of supplemental dietary protein content on balances and apparent digestibilities of macrominerals in Jersey cows.

Item	Dietary CP content, % DM 11	15	19	SEM	P[a]
	----------(g/d)----------				
Ca intake	27.8	28.8	31.1	.37	<.01
Fecal Ca	31.5	30.6	32.7	1.10	<.45
Urine Ca	.12	.13	.20	.03	<.11
Ca balance	-3.85	-1.88	-1.86	1.08	<.24
Ca digestibility, %	-14.5	-6.40	-3.70	4.09	<.19
P intake	18.8	19.8	20.5	.12	<.01
Fecal P	16.8	17.8	18.0	1.14	<.49
Urine P	1.5	2.3	2.0	.40	<.37
P balance	.5	-.4	.5	.72	<.97
P digestibility, %	11.3	9.6	12.6	5.13	<.86
Mg intake	11.4	12.6	13.5	.08	<.01
Fecal Mg	2.9	3.2	3.3	.18	<.16
Urine Mg	8.8	9.6	10.6	.15	<.01
Mg balance	-.3	-.3	-.4	.16	<.67
Mg digestibility, %	22.4	23.9	21.2	1.38	<.56
S intake	8.0	14.8	23.8	.26	<.01
Fecal S	6.4	9.9	13.8	1.14	<.01
Urine S	4.3	7.6	11.3	.51	<.01
S balance	-2.7	-2.7	-1.3	.87	<.29
S digestibility, %	20.2	33.1	41.7	8.23	<.13

[a]Probability for linear effect of supplemental dietary CP content; no evidence of curvilinearity.

Table 3-6. Effects of supplemental dietary protein content on fractional excretion rate of macrominerals.[1]

Item	Dietary CP content, % DM 11	15	19	SEM	P[a]
	----------(%)----------				
Ca	.09	1.46	1.60	.35	<.32
P	1.80	2.15	2.41	.53	<.95
Mg	11.74	12.69	11.67	.83	<.95
S	21.01	25.50	23.99	3.00	<.54

[a]Probability for linear effect of supplemental dietary CP content; no evidence of curvilinearity.

[1]Fractional excretion rate = [(urine mineral concentration/plasma mineral concentration) X (plasma creatinine concentration/urine creatinine concentration)] x 100.

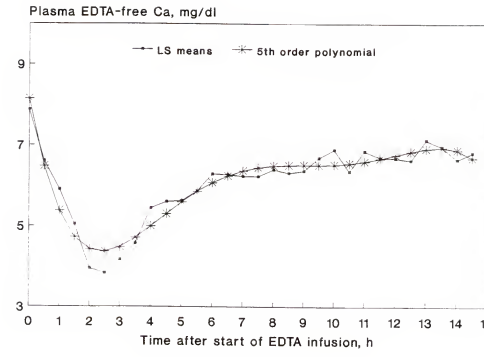

Figure 3-1. Comparison between least squares means and fifth order polynomial curve for EDTA-free Ca concentrations over sampling times. The values for each time point were pooled across all treatments. The infusion of EDTA started at 0 h and ceased at approximately 2.5 h.

EDTA-free Ca, total plasma Ca and EDTA-bound Ca at different sampling times after commencing EDTA infusion are presented in Figure 3-2, Figure 3-3 and Figure 3-4, respectively. For the purpose of comparison, the least squares means of EDTA-free Ca, total Ca and EDTA-bound Ca are presented together in Figure 3-5.

Discussion

Increased protein intake affected metabolism of Ca by humans (Yuen, 1984). However, a similar effect might not be expected with ruminants if the additional supplemental protein was highly degradable in the rumen. Therefore, to increase the likelihood of more supplemental protein escaping rumen degradation, hydrolyzed feather meal (.70 undegradability) and distillers dried grains with solubles (.47 undegradability) were used as supplemental protein sources (NRC, 1989). Concomitantly, because of the high S content of feather meal (ie., 1.91% S, DM basis) total dietary S increased with increasing protein source supplementation. Thus, effects potentially attributable to supplemental protein per se were not intended to be differentiated from effects possibly due to S in the present experiment.

A mild acidosis was induced in cows fed increased amounts of supplemental protein sources as shown by a decrease in blood base excess, a lower excretion of urinary bases, and elevated urinary ammonium (Table 3-4). This agrees with

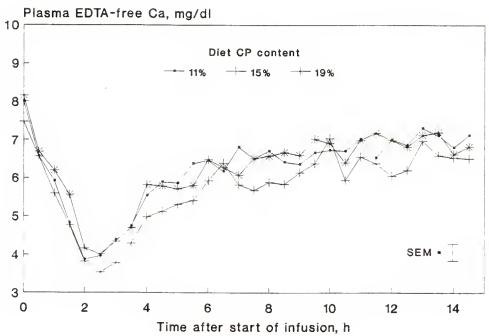

Figure 3-2. Least squares means of plasma EDTA-free Ca concentrations at different sampling times. The infusion of EDTA started at 0 h and ceased at approximately 2.5 h.

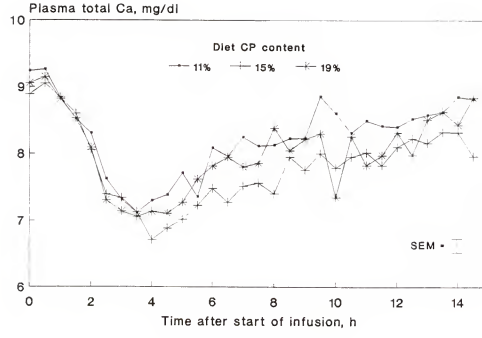

Figure 3-3. Least squares means of plasma total Ca concentrations at different sampling times. The infusion of EDTA started at 0 h and ceased at approximately 2.5 h.

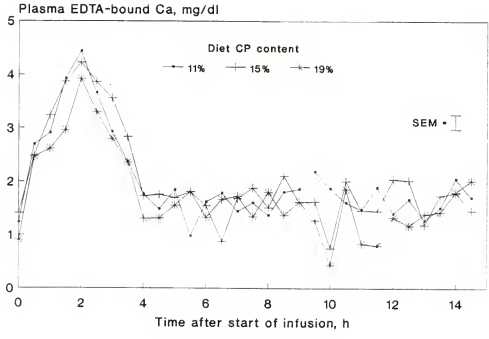

Figure 3-4. Least squares means of plasma EDTA-bound Ca concentrations at different sampling times. The infusion of EDTA started at 0 h and ceased at approximately 2.5 h.

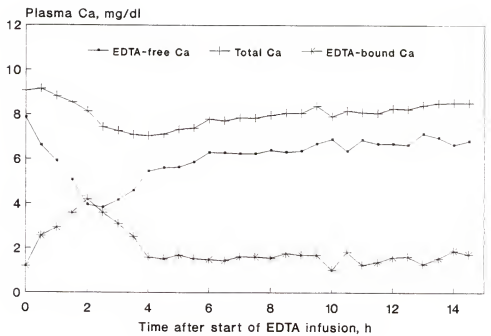

Figure 3-5. Least squares means of EDTA-free Ca, total Ca and EDTA-bound Ca at different sampling times. The values for each time were pooled across all treatments. The infusion of EDTA started at 0 h and ceased at approximately 2.5 h.

results observed with other species (Petito and Evans, 1984), and partially could be accounted for by oxidation of SAA resulting in sulfate and hydrogen ions being excreted in urine (Hunt, 1956). The SAA concentration of the 15 and 19% CP diets in this study was increased by the substitution of hydrolyzed feather meal and distillers dried grains for part of the ground corn. When DL-methionine-supplemented liquid diets were fed to young men for several days, the accumulative increments in S excretion virtually were equal to the accumulative increments in urinary net acid excretion (Lemann and Relman, 1979).

No differences were found in apparent digestibility or balance of Ca among treatments. Calcium balance was negative for all cows in this experiment. This was because more endogenous Ca apparently flowed into the gut lumen than actually was absorbed (Table 3-5). Endogenous Ca excreted into the gut lumen might reach 8 to 12 g (Moodie, 1960). In older cows, endogenous fecal Ca remains similar to that of younger cows, but Ca is absorbed less efficiently, and negative Ca balance occurs even in nonlactating cows (Moodie, 1960).

Calcium excreted in urine was only .47% of total Ca excretion by cows in this study when averaged over all treatments. This was similar to findings by others (Moodie, 1960), showing that urinary Ca excretion was 1 g/d or less in cows. Paquay et al. (1968) also reported that urinary Ca

excretion ranged between .2 and 6.7 g/d in dry cows. Urinary excretion is a minor route of Ca excretion in cows. The bovine kidney is highly efficient in conserving Ca and 97% of glomerularly filtered Ca was reabsorbed (Ramberg et al., 1975). On average, almost 99% of Ca filtered by the kidney was reabsorbed in our study. Urinary excretion of Ca in cattle apparently does not vary significantly as it does in humans, where the kidney is considered as a major regulator of Ca balance and plasma Ca concentration (Ramberg et al., 1975).

The EDTA chelates Ca and makes it physiologically unavailable. Calcium bound to EDTA is excreted into urine, resulting in decreased plasma Ca concentrations (Aronson and Ahrens, 1971; Forman et al., 1954). Calcium in the interstitial fluid may be drawn to blood by exchange within minutes (Moodie, 1960). In the present study, cows were not fed on the morning of EDTA infusion. Thus, the contribution of Ca absorbed from the gut was probably small. The recovery of concentration of plasma Ca might have depended mainly on Ca mobilized from bone. Concentrations of EDTA-free Ca and total plasma Ca approached preinfusion concentrations by 7 h after the end of the infusion (Figures 3-2 and 3-3), suggesting that Ca mobilization from bone occurred for cows on all treatments.

Responses to EDTA infusion were used by several researchers to estimate Ca mobilization rate. However, the procedures used and the interpretation of results differed (Van de Braak et al., 1984). Assumptions used in calculating

Ca pool and Ca mobilization rate from Ca concentration changes in response to EDTA infusion were questioned. Furthermore, results of the calculations were dependent on the rate of infusion and amount of EDTA infused. A sufficiently severe hypocalcemia induced with a constant infusion rate and common end point is necessary for any meaningful comparisons among treatment groups. Van de Braak et al. (1984) proposed infusing EDTA until clinical signs of hypocalcemia occur and using the amount of EDTA infused to each cow, instead of Ca pool or Ca mobilization rate calculated indirectly, to evaluate the cow's ability to mobilize Ca. In our experience, determining the appropriate end point of EDTA infusion was difficult because assessment of the severity of clinical signs was somewhat subjective and variable among cows. Therefore, we elected to infuse cows within each square simultaneously until one of the cows showed signs of severe hypocalcemia. In this way, a sufficiently severe hypocalcemia was induced, and the endpoint of infusion was the same for all the cows in that square. All other conditions were the same. The effect of individual cows was accounted for in the Latin square design (cow was included as an independent source of variation in the mathematical model). Changes in plasma Ca concentrations over sampling time were best estimated by a 5th degree polynomial. Tests of heterogeneity of the regression were used to test for nonparallelism among the regression curves for treatments. In this study, there was no evidence ($P > .20$) curves for the

three treatments were not parallel, indicating the bone mobilization rate was not affected by supplemental protein or S. Cows in our study consumed relatively small amounts of Ca (29 g/d). With higher intake of Ca, bone resorption was minimal (Yarrington et al., 1977). Whether the response would be different with higher Ca intake could not be determined from our study.

Metabolic acidosis caused increased bone mobilization in rats and goats (Barzel, 1969; Fredeen et al., 1988a, b). The acidosis induced in our study was relatively mild. Daily consumption of S was about 24 g/d for the cows fed the 19% CP diet. This was lower than that in the study of Oetzel et al. (1988), who found that feeding 24 g of S from ammonium sulfate, in addition to 6 g of S contained in the basal diet plus 100 g of NH_4Cl, reduced incidence of hypocalcemia. If the effect of protein supplements depended totally on S content, protein supplements at concentrations used in our study did not provide enough S. However, a higher intake of protein than that used in this study probably would be impractical or uneconomical to use for dry cow rations.

Diets with negative fixed cation-anion difference were shown to reduce incidences of PP (Block, 1984; Dishington et al., 1975). Cation-anion difference calculated as milliequivalents of (Na + K) - (Cl + S) were 269, 210 and 147/kg DM for 11, 15, 19% CP diets, respectively (Table 3-2). Protein supplementation did not create negative dietary

cation-anion difference in this experiment. This also may help to explain why Ca metabolism was not affected.

In conclusion, increasing supplemental dietary protein intake induced mild acidosis but had no effect on Ca balance or responses to intravenous infusion of EDTA solution under conditions of this study. Urinary Ca excretion was a very small fraction of total Ca excretion in dairy cows. Sulfur excretion in both urine and feces increased as S intake increased with increased supplemental protein intake.

CHAPTER 4
EFFECTS OF AMMONIUM CHLORIDE AND AMMONIUM SULFATE ON ACID-BASE STATUS AND CALCIUM METABOLISM OF NONLACTATING JERSEY COWS

Introduction

Parturient paresis (PP) is a metabolic disorder characterized by the inability of dairy cows to meet the demand for Ca at parturition (Ramberg et al., 1975). Alkaline diets induced high incidence of PP, but diets with added mineral acids (HCl and H_2SO_4) prevented PP in dairy cows (Ender et al., 1962). A mixture of $CaCl_2$, $MgSO_4$ and $Al_2(SO_4)_3$ added to dry cow diets also prevented PP in Norway (Dishington et al. 1975). A similar mixture of these salts prevented PP in a switch-over trial (2 yr) with 20 cows in Canada (Block, 1984). The effects of these salts were attributed to the reduction of dietary cation-anion difference (DCAD) calculated as (Na + K) - (Cl + S) in meq/kg diet DM (Ender et al., 1962; Dishington, 1975; Block, 1984).

Other compounds also were tested for their effects on Ca metabolism and PP prevention. Ammonium chloride induced metabolic acidosis and increased bone mobilization (Kendall et al., 1969; Vagg and Payne, 1970). The effects on incidence of PP were inconclusive because of the small number of cows used in these studies. Horst and Jorgensen (1973) found that NH_4Cl depressed feed intake severely when given in capsules to

goats. It was stated that NH_4Cl was an unlikely agent for PP prevention, because it depressed feed intake severely and may cause toxicity (Jorgensen, 1974). Recently, Oetzel et al. (1988) showed that a mixture of 100 g NH_4Cl plus 100 g $(NH_4)_2SO_4$ in a total mixed diet fed prepartum decreased the risk of hypocalcemia (blood ionized Ca < 4mg/dl) with an insignificant decrease in incidence of PP with 48 Holstein cows. The objective of the present study was to study further the effects of the mixture of ammonium salts on acid-base status, calcium metabolism and the responses to hypocalcemia induced by intravenous infusion of Na_2EDTA solution.

Materials and Methods

Eight nonlactating, nonpregnant multiparous Jersey cows (age 71 ± 15 mo) were assigned randomly to treatment sequences in a cross-over design with two periods of 28 d each. The cows were blocked into two groups according to initial BW. Cows of group 1 averaged 397±23 kg (mean±SD) and cows of group 2 averaged 472±23 kg (mean±SD) at the beginning of the experiment. The control diet (Control) consisted of corn silage, ground corn, soybean meal, and supplements of minerals and vitamins with a silage to concentrate ratio of 68:32 on a DM basis (Table 4-1). The treatment diet (Ammonium salts) was the same as the Control except NH_4Cl and $(NH_4)_2SO_4$ were added to the concentrate to provide 98 g of each/cow/d. All eight cows were kept in individual tie stalls in a ventilated flat

Table 4-1. Ingredient composition of experimental diets fed to Jersey cows.

Ingredients	Control	Ammonium salts
	----------(% DM)----------	
Corn silage	68	68
Ground corn	26.1	24.9
Soybean meal, 48% CP	2.2	2.2
Trace mineral salt[1]	.30	.30
Ammonium sulfate	...	1.15
Ammonium chloride	...	1.15
Urea	1.10	...
Calcium carbonate	2.30	2.30
Vitamin A, IU/kg	3960	3960
Vitamin D, IU/kg	1188	1188
Vitamin E, IU/kg	22	22

[1]Composed of 95 to 99% NaCl, .35% Zn, .34% Fe, .20% Mn, .033% Cu, .007% I, and .005% Co.

barn with free access to drinking water. Cows were allowed to exercise for 4 h in an open lot twice weekly. They were fed rations in two equal amounts at 0800 and 1400 h daily. During a 14 d preliminary period, the cows were fed the Control diet. The daily feed intake was adjusted to the amount (8.5 kg DM/d) that all cows would consume and kept the same throughout the experiment.

Samples of concentrates were taken weekly and pooled within cow-period. Samples of corn silage also were taken weekly and dried in 60^0C for 72 h. The DM content was used to adjust the concentrate-to-corn silage ratios weekly. Daily individual water intake was measured by in-line flow meters. Cows were weighed on a digital electronic scale at the beginning and end of each period. Before the 0700 h feeding on d 25, 26 and 27 of each period, urine samples (500 ml) were collected by vulva stimulation. Urine pH was determined immediately. One sample with 1 ml concentrated HCl in 9 ml urine and one unacidified were stored frozen (-10^0C) in plastic test tubes until analyzed. One blood sample was taken anaerobically from the jugular vein and analyzed immediately for blood ionized Ca (iCa) and pH. Another 25-ml blood sample was taken into two 14-ml plastic tubes coated with ammonium heparin (20 U/ml). After centrifugation at 2800 X g for 20 min, plasma was harvested and stored in small plastic tubes until analyzed for creatinine, Ca, Mg and P.

On the afternoon of d 27 of each period, catheters were inserted into both jugular veins and kept patent with saline (.99% NaCl) solution containing 100 U/ml ammonium heparin. On d 28, a 10% saline solution of Na_2EDTA (pH adjusted to 7.4 with NaOH) was infused simultaneously into four cows within one block at an average rate of 3.1 ml/min (3.1 mg/min or .43 mg/h/kg BW). Blood samples were taken immediately before the start of the infusion and at 30 min intervals for 14.5 h after the start of the Na_2EDTA infusion. At each sampling point, one sample was taken anaerobically and analyzed immediately for iCa and pH. Another 25 ml of blood was taken into 30 ml syringes and transferred to two 14 ml plastic tubes coated with ammonium heparin (20 Units/ml). After centrifugation at 2800 X g for 20 min, plasma was harvested and stored in small plastic tubes until analyzed.

In this study, the endpoint of EDTA infusion was different from that of the first study (Chapter 3). When one of the 4 cows in an infusion session showed inability to stand, blood samples were taken simultaneously from all four cows. The infusion continued for each of the remaining cows until she showed inability to stand and other signs of severe hypocalcemia, such as excessive salivation, teeth-grinding and trembling.

Feed samples were ground through 2-mm screen (Thomas-Wiley Mill, Model 4, Thomas Scientific, Philadelphia, PA). Feed DM was determined by drying at 100^0C for 24 h. Feed

samples were ashed at 550 ^{0}C for 6 h. After dissolving the ash in 3 N HCl, Ca, Mg, Na, and K were determined by atomic absorption spectrophotometry (Model 5000, Perkin Elmer, Inc., Norwalk, CT). Phosphorus was determined by colorimetry (Fiske and Subbarow, 1925). Dietary Cl was determined by potentiometric titration (Haake Buchler Instruments, Inc., Saddlebrook, NJ.). For plasma and urine samples, the same methods used for feed samples were used to determine concentrations of total Ca, Mg and P after the samples were deproteinized with 10% TCA. Concentrations of plasma and urine creatinine were determined according to Henry et al. (1974).

Another major difference in protocol between the first experiment (Chapter 3) and this experiment was that in the present study blood iCa and pH were determined immediately on a NOVA 8 Analyzer (NOVA Biochemicals, Waltham, MA). Urine pH was determined daily on subsamples. Titratable acidity and ammonium concentrations were determined by titration of non-acidified urine samples (Chan, 1972; Lin and Chan, 1973). Net acid concentrations were the sum of urine titratable acidity and ammonium. Concentrations of minerals and creatinine in plasma and urine were used to calculate fractional renal excretion of minerals ([(urine mineral/plasma mineral) X (plasma creatinine/urine creatinine)] X 100) (Lane and Merritt, 1983).

Statistical analyses were performed by method of least squares ANOVA, using general linear model procedures of SAS (Barr et al., 1982). For dependent variables with one observation in each cow-period cell, block, cow within block, period, treatment, period by block interaction, treatment by block interaction were in the mathematical model. For variables with observations on three separate days within each cow-period, day, and interactions of day with all other terms were included in the model. Blood concentrations of iCa, plasma total Ca and Mg after the start of EDTA infusion were estimated by polynomial regressions up to the fifth order, considering time as a continuous independent variable. Tests for heterogeneity of regression were performed to examine the effects of treatments over time after the start of EDTA infusion (Snedecor and Cochran, 1980).

Results

Table 4-2 shows the chemical composition of the experimental diets. In the diet supplemented with ammonium salts, DCAD calculated as milliequivalents [(Na + K) - (Cl + S)] per 1000 g DM was more negative than that in the control diet due to the additional Cl and S provided by NH_4Cl and $(NH_4)SO_4$. Water intake was similar for both the control and the treatment groups, averaging 14 L/d. Feed intake was the same for both the treatment and control groups, because cows were fed a fixed amount of ration throughout the experiment.

Table 4-2. Laboratory analysis of chemical composition (dry basis) of experimental diets fed to Jersey cows.

Item	Control	Ammonium salts
DM, %	48.2	48.2
NE_l, Mcal/kg[1]	1.65	1.65
CP, %	13.8	14.4
ADF, %	20.1	20.4
Ca, %	1.40	1.44
P, %	.24	.24
Mg, %	.20	.20
Na, meq/kg	55	52
K, meq/kg	250	250
Cl, meq/kg	124	371
S, meq/kg	112	359
(Na+K)-(Cl+S), meq/kg	69	-428

[1]Calculated from values of individual feedstuffs listed by NRC (1989).

Body weights increased by 8.6 kg/cow during each period similarly for both the treatment and control groups.

Both blood pH and urine pH were lower for the treatment group than for the control group ($P < .01$; $P < .01$) (Table 4-3). Urinary titratable acidity, ammonium concentration, net acid concentration, and their creatinine ratios were much higher for the Ammonium salt group than for Control group ($P < .01$) (Table 4-3).

The blood concentration of ionized Ca was higher for the treatment group than for the control group ($P < .05$) (Table 4-4). However, concentrations of plasma total Ca, Mg and P were not different between the treatment group and the control group (Table 4-4). Urine Ca/creatinine ratio, but not P/creatinine ratio was higher for the treatment group than for the control group ($P < .01$) (Table 4-5). Urine Mg/creatinine ratio tended to be lower in the treatment group ($P < .15$). These were accompanied by higher fractional excretion of Ca ($P < .01$) and a lower fractional excretion of Mg ($P < .08$) in the treatment group (Table 4-5).

The rate of Na_2EDTA infusion was similar for both the treatment and control groups. The infusion time ($P < .14$) and total volume of EDTA solution ($P < .16$) required to cause severe hypocalcemia tended to be higher for the Ammonium salts group than for control group (Table 4-6). After an equal volume of EDTA solution was infused (when the first cow showed inability to stand), blood iCa was higher but blood pH was

Table 4-3. Effects of diets supplemented with ammonium chloride and ammonium sulfate on acid-base status of Jersey cows.

Item	Control	Ammonium salts	SEM	P
Blood pH	7.46	7.38	.01	<.01
Urine				
pH	7.88	5.93	.10	<.01
TA[1], meq/L	-60.77	2.73	4.21	<.01
NH_4, meq/L	8.58	65.73	8.31	<.01
NAC[2], meq/L	-52.20	68.47	9.79	<.01
TA/Cr[3]	-15.32	.57	3.30	<.03
NH_4/Cr[3]	1.87	14.56	1.46	<.04
NAC/Cr[3]	-13.45	15.16	3.57	<.01

[1]Titratable acidity equals the amount of NaOH required to titrate urine to pH 7.4.

[2]Net acid concentration equals titratable acidity plus ammonium.

[3]TA/creatinine ratio, ammonium/creatinine ratio and net acid concentration/creatinine ratio, mmol/mmol.

Table 4-4. Effects of diets supplemented with ammonium chloride and ammonium sulfate on concentrations of blood ionized calcium and plasma total calcium, magnesium and phosphorus of Jersey cows.

Item	Control	Ammonium salts	SEM	P
Blood iCa, mg/dl	4.68	4.88	.04	<.02
Plasma				
Ca, mg/dl	9.64	9.56	.08	<.51
Mg, mg/dl	2.19	2.28	.05	<.19
P, mg/dl	5.70	5.76	.06	<.63

Table 4-5. Effects of diets supplemented with ammonium chloride and ammonium sulfate on urinary excretion of calcium, magnesium and phosphorus in Jersey cows.

Item	Control	Ammonium salts	SEM	P
Ca/Cr[1]	.46	3.02	.19	<.01
Mg/Cr[1]	3.86	2.98	.35	<.15
P/Cr[1]	.51	.78	.13	<.22
Ca FE[2], %	1.55	10.09	.68	<.01
Mg FE[2], %	33.34	24.72	2.65	<.08
P FE[2], %	1.63	2.40	.34	<.18

[1]Ca/creatinine ratio, Mg/creatinine ratio and P/creatinine ratio, mmol/mmol.

[2]Fractional excretion of Ca = [(urine Ca/plasma Ca) X (plasma creatinine/urine creatinine)] X 100. Similar calculation for fractional excretion of Mg and P.

Table 4-6. Parameters of EDTA infusion and changes in blood concentrations of ionized calcium and pH of Jersey cows.

Item	Control	Ammonium salts	SEM	P
Volume, ml	526	624	38	<.14
Time, min	172	204	13	<.16
Rate, ml/min	3.05	3.06	.02	<.65
	--after equal amount of EDTA infused--			
Blood iCa, mg/dl	2.12	2.44	.08	<.04
Blood pH	7.42	7.34	.02	<.02

lower for the ammonium salts group than for the control group ($P < .05$).

Blood pH was lower for the ammonium salts group at all sampling times (Figure 4-1). Blood concentrations of Ca decreased drastically when EDTA solution was infused, and then recovered gradually after the infusion stopped (Figure 4-2, 4-3). Blood concentrations of iCa and plasma concentrations of total Ca were estimated by polynomial regressions to the fifth order. Test of heterogeneity of regression showed that the curves for the two groups were not parallel for iCa ($P < .01$) or plasma total Ca ($P < .01$). Figure 4-2 and Figure 4-3 show, respectively, the least squares means of concentrations of blood iCa and plasma total Ca at different times after the start of EDTA infusion.

Plasma concentrations of Mg at different times after start of the EDTA infusion were estimated by polynomial regressions of the fourth order. Test of heterogeneity of regression showed that the curves for the ammonium salts group and the control group were not parallel ($P < .01$). Figure 4-4 shows the least squares means of plasma Mg concentrations at different times after the start of EDTA infusion.

Discussion

In the present experiment, cows were fed a fixed amount of ration throughout the experiment. Thus, the effects of variation in feed intake were eliminated. The effects due to

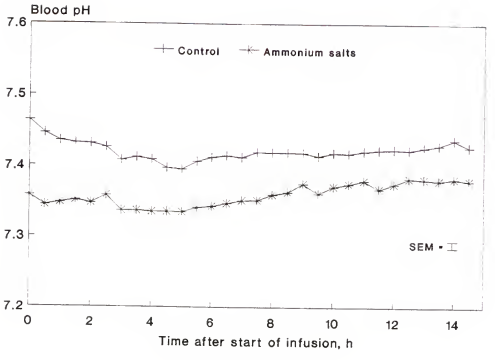

Figure 4-1. Least squares means of blood pH of Jersey cows at different sampling times. The infusion of EDTA started at 0 h and ceased at approximately 3.0 h.

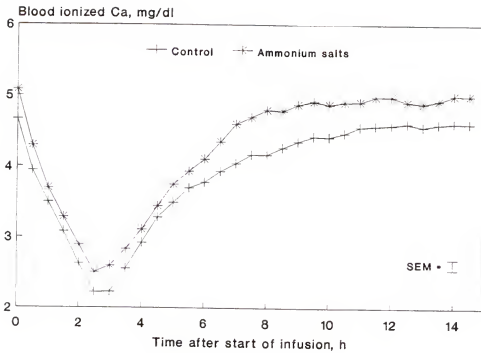

Figure 4-2. Least squares means of blood ionized Ca concentrations of Jersey cows at different sampling times. The infusion of EDTA started at 0 h and ceased at approximately 3.0 h.

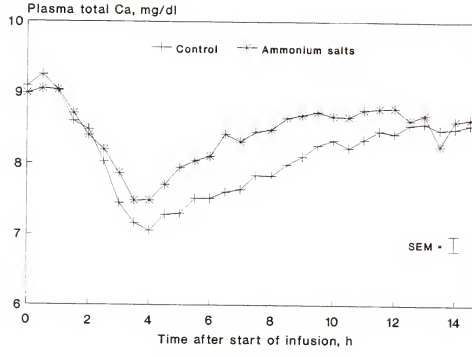

Figure 4-3. Least squares means of plasma total Ca concentrations of Jersey cows at different sampling times. The infusion of EDTA started at 0 h and ceased at 3.0 h.

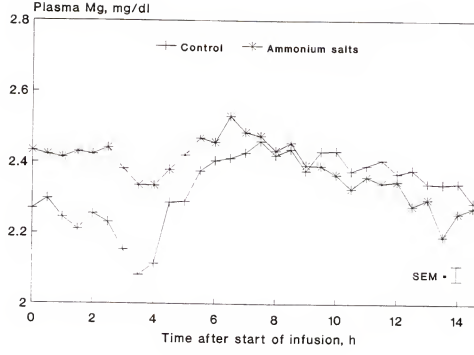

Figure 4-4. Least squares means of plasma Mg concentrations of Jersey cows at different sampling times. The infusion of EDTA started at 0 h and ceased at approximately 3.0 h.

cow to cow variation could be controlled to some extent statistically because of the switchover design used. The effects of parturition, health and management factors associated with parturition did not exist in this study because nonlactating and nonpregnant cows were used. The metabolic effects of NH_4Cl and $(NH_4)_2SO_4$, might be estimated better than in a continuous experiment with pregnant cows.

Laboratory analysis of the diets showed that the treatment diet had much higher concentrations of Cl and S due to the supplementation of the ammonium salts. The CP content of the two diets was similar because urea was added to the control diet to equal the amount of N added to the treatment diet from the ammonium salts. Only during the initial 1 to 3 d of each period, cows on the treatment diet did not completely consume all the ration offered. It was noticed that cows fed the ammonium salts took more time than cows fed the control diet to completely consume the feed. This suggests that diets with ammonium salts were less palatable, as noted by Horst and Jorgensen (1973). This may not be a problem considering that dry cows are usually fed at close to maintenance level of intake. Cows on this study were fed slightly above maintenance requirement as shown by the increase in BW during the experiment.

Blood pH was within the physiologically normal range for both groups, but was lower ($P < .01$) for the ammonium salts group compared with the control group, indicating that dietary

supplementation of the ammonium salts induced a subclinical metabolic acidosis in the ammonium salts group. Blood pH were lower for the cows fed the ammonium salts than for the control group during EDTA infusion and the recovery period as well (Figure 4-1). The lower urine pH, increased concentrations of urinary titratable acidity, ammonium and net acid concentration reflected the compensatory responses by the kidney. These results were in agreement with others (Kendall et al., 1969; Vagg and Payne, 1970).

Calcium is present in the plasma in three major forms: ionized, protein-bound and complexed. The ionized form is the biologically active form, representing about 50% of the total plasma Ca (Ender et al. 1962). In the present study, the blood iCa concentration was increased, but the total plasma Ca concentration was not affected by the ammonium salts fed in the treatment diet. The ratio of iCa to total Ca was .51 for the ammonium salts group, compared with .48 for the control group. Others have reported increased concentrations of total plasma Ca in dairy cows (Block, 1984), and in goats (Fredeen et al., 1988a,b), when acidogenic diets were fed. Oetzel et al. (1988) showed that diets supplemented with a mixture of NH_4Cl and $(NH_4)_2SO_4$ increased plasma concentrations of both ionized Ca and total Ca, but not the ratio of the two Ca forms around parturition. Blood pH was not reported by Oetzel et al. (1988). Thus, it is difficult to assess to what extent the blood pH was changed in that study. In our study blood pH

supplementation of the ammonium salts induced a subclinical metabolic acidosis in the ammonium salts group. Blood pH were lower for the cows fed the ammonium salts than for the control group during EDTA infusion and the recovery period as well (Figure 4-1). The lower urine pH, increased concentrations of urinary titratable acidity, ammonium and net acid concentration reflected the compensatory responses by the kidney. These results were in agreement with others (Kendall et al., 1969; Vagg and Payne, 1970).

Calcium is present in the plasma in three major forms: ionized, protein-bound and complexed. The ionized form is the biologically active form, representing about 50% of the total plasma Ca (Ender et al. 1962). In the present study, the blood iCa concentration was increased, but the total plasma Ca concentration was not affected by the ammonium salts fed in the treatment diet. The ratio of iCa to total Ca was .51 for the ammonium salts group, compared with .48 for the control group. Others have reported increased concentrations of total plasma Ca in dairy cows (Block, 1984), and in goats (Fredeen et al., 1988a,b), when acidogenic diets were fed. Oetzel et al. (1988) showed that diets supplemented with a mixture of NH_4Cl and $(NH_4)_2SO_4$ increased plasma concentrations of both ionized Ca and total Ca, but not the ratio of the two Ca forms around parturition. Blood pH was not reported by Oetzel et al. (1988). Thus, it is difficult to assess to what extent the blood pH was changed in that study. In our study blood pH

was reduced ($P < .01$) by the ammonium salts, compared with the control (Table 4-3). The changes in blood pH were most likely responsible for the changes in blood Ca concentrations. This was supported by research of Moore (1970), who examined the effect of pH on iCa concentration over a pH range of 6.8 to 7.8 with human blood in vitro. He demonstrated instantaneous and completely reversible changes in iCa concentration in response to variation of pH. The blood concentration of iCa increased in a linear or slightly sigmoid fashion with decreasing pH. He suggested that the effect of pH on the iCa concentration may be due to competition between Ca and H^+ for binding sites on serum proteins. The increased concentration of blood iCa in acidemic cows may contribute to the maintenance of blood Ca concentration when the demand for iCa is suddenly increased.

In general, bovine kidney is highly efficient in conserving Ca (Ramberg et al., 1975). Similar to our previous study (Wang and Beede, 1990), more than 98% of Ca filtered by the kidney was reabsorbed in the control group (Table 4-5). However, that the amount of Ca excreted into urine is usually small in cattle may not necessarily mean low capacity of bovine kidneys for handling Ca. Urinary Ca excretion increased markedly when cows were given i.v. infusion of Ca solution (Ramberg et al., 1975). In our study, about 10% of filtered Ca was excreted by cows fed the ammonium salts, compared with less than 2% by cows fed the control diet.

Fredeen et al. (1988b) also found an increase in urine Ca excretion in goats fed diets with HCl. These results were in agreement with Stacy and Wilson (1970), who showed that sheep responded to acidosis by decreasing the tubular reabsorption of filtered Ca.

Normal plasma Mg concentrations range from .85 to 1.20 mmol/L (Sansom et al., 1983). In the present study, plasma Mg concentrations for both the treatment and control groups were within the normal range (Table 4-4). It was noted that urine Mg excretion tended to be lower for the ammonium salts group, as shown by a lower Mg/creatinine ratio and fractional excretion rate of Mg (Table 4-5). The reason for this was not clear. Acidosis increased Mg excretion in other species (Nichols and Tannen, 1987) and in ruminants (Fredeen et al., 1988b). Dishington (1975) even observed hypomagnesemia in some cows fed acidogenic diets. High Ca intake may decrease Mg absorption (Alcock and MacIntyre, 1962; Verdaris and Evans, 1976). In this experiment, Ca intake was relatively high (120.7 g/d). The lower urinary Mg excretion could be reflecting the reduced Mg absorption due to this high Ca intake. Others also observed that hypercalciuria might occur without hypermagnesemia in sheep made acidemic by intravenous infusion of HCl (Stacy and Wilson, 1970).

The responses to EDTA infusion were used previously by several researchers and ourselves to estimate the cows' ability to maintain normal blood Ca concentrations when Ca

demand was suddenly increased, simulating the metabolic events at parturition (Contreras et al., 1981; Van de Braak et al., 1984; Wang and Beede, 1990). In the present study, results from EDTA infusion showed that cows in the treatment group were more capable of maintaining normal blood Ca concentrations when the demand for Ca was suddenly increased by EDTA infusion. After an equal volume of EDTA solution was infused into all cows simultaneously, cows in the treatment group maintained a higher concentration of blood iCa. It took a larger volume of EDTA solution and a longer infusion time for Cows in the treatment group to show severe signs of hypocalcemia (inability to stand). Concentrations of blood iCa, as well as total plasma Ca for the ammonium salts group recovered more rapidly than the control group (Figure 4-2, 4-3).

Plasma Mg concentrations were unaffected by intravenous infusion of Na_2EDTA in 21 Ayrshire cows (Payne, 1964). Ramberg et al. (1967) did not observe changes in plasma Mg concentrations of 8 cows with hypocalcemia induced by Na_2EDTA infusion. Further study by Daniel (1980) showed that plasma Mg concentrations of cows infused with Na_2EDTA were not different from those of cows infused with saline solution, although plasma Ca concentrations decreased in parallel with plasma Ca in wethers infused with Na_2EDTA solution. It was indicated that EDTA combined with Ca preferentially when infused into the blood, even though it could also bind Mg

(Popovici et al. 1950). In the present study, the plasma Mg concentration showed slight changes over time after the start of EDTA infusion of EDTA in all cows. However, plasma Mg concentration tended to be higher in cows fed the ammonium salts (Figure 4-4).

Our results imply that supplementation of the ammonium salts resulted in an increased input of iCa into the blood Ca pool. Total input of iCa into the blood Ca pool depends mainly on Ca absorbed from the intestine and Ca mobilized from bone (Ramberg et al., 1975). Whether feeding these ammonium salts affected Ca absorption in this study was not clear. Other studies with other acidogenic salts ($CaCl_2$, $Al_2(SO_4)_3$ and $MgSO_4$) provided some clues. Ender and Dishington (1970) showed that feeding prepartum diets supplemented with acidogenic salts increased Ca absorption with a resulting positive Ca balance around parturition. Acidogenic diets increased Ca absorption in sheep (Braithwaite, 1972; Fredeen et al., 1988a,b). Lomba et al. (1978) summarized data from a number of experiments, indicating that acidogenic diets increased Ca absorption only when cows were on positive Ca balance. This implied that negative DCAD increased Ca absorption only when Ca intake was high. However, Leclerc and Block (1989) did not find any difference in apparent Ca absorption among cows fed diets with DCAD of 349, 121, 105 or 62 meq/kg DM during the dry period with Ca intake of 150 to 190 g/cow/d. The diets with less positive DCAD resulted in

slightly reduced apparent Ca absorption at parturition. It seems that the effect of negative DCAD on Ca absorption may be relatively small. In the present study, during the infusion period and the subsequent sampling period, cows were not fed. The amount of Ca absorbed from the intestine may be minimal. Increased Ca mobilization from bones may be the key mechanism for resisting the effect of EDTA and in returning blood Ca to normal after the end of EDTA infusion. Acidosis increases Ca mobilization from bone by releasing Ca from amorphous Ca phosphates and Ca carbonates on bone surfaces (Termine and Posner, 1966; Bushinsky et al., 1985). Acidosis also may increase cell-mediated bone resorption (Mundy and Raisz, 1981). Other studies have showed that plasma hydroxyproline, an indicator of bone resorption, was increased in cows fed acidogenic diets (Block, 1984). Fredeen et al., (1988b) recently showed that an acidogenic diet increased bone resorption rate measured by ^{45}Ca kinetics studies. They suggested that acidogenic diet increased the turnover rate of the available Ca pool. Vagg and Payne (1970) indicated that the size of Ca pool also may be increased by acidogenic diets.

Results of this study showed that supplementation of NH_4Cl and $(NH_4)_2SO_4$ induced metabolic acidosis, increased the rate of Ca mobilization and the cows' ability to maintain blood iCa and plasma total Ca concentrations close to normal when the demand for Ca was increased suddenly.

CHAPTER 5
EFFECTS OF DIETARY MAGNESIUM INTAKE ON ACID-BASE STATUS AND CALCIUM METABOLISM OF NONLACTATING HOLSTEIN COWS FED ACIDOGENIC SALTS

Introduction

Parturient paresis (PP) is a metabolic disorder characterized by the inability of cows to meet the increased demand for Ca at parturition (Ramberg et al., 1975). Ender et al. (1962) in Norway first found that PP was induced by diets with beets rich in Na and K, but prevented by diets with silage preserved with mineral acids (HCl and H_2SO_4), regardless of Ca and P intake. The opposite effects of the two types of diets were verified by feeding experiments with ordinary diets supplemented with either alkalogenic salts (Na_2CO_3 + $NaHCO_3$) or acidogenic salts ($CaCl_2$, $Al_2(SO_4)_2$ plus $MgSO_4$), to match the contents of Na, K, Cl and S in beets and silages used previously (Dishington, 1975). Out of 14 cows fed Na_2CO_3 + $NaHCO_3$, 13 suffered from PP. Twelve of 13 cows fed $CaCl_2$, $Al_2(SO_4)_2$ plus $MgSO_4$ were healthy with only one cow showing mild symptoms of PP. The effectiveness of the acidogenic salts also were shown by others (Dishington and Bjornstad, 1982; Block, 1984). The preventive effects of these diets were attributed to the negative cation-anion difference (DCAD) calculated as [(Na + K) - (Cl + S)] in meq/kg diet DM.

Another acidogenic salt, NH_4Cl, also induced acidosis and increased bone resorption in ruminants (Vagg and Payne, 1970). However, NH_4Cl first was considered practically unsuitable for PP prevention, because it severely depressed feed intake and might cause toxicity (Jorgensen, 1974). Recently, Oetzel et al. (1988) showed that a mixture of 100 g NH_4Cl plus 100 g $(NH_4)_2SO_4$ in a total mixed prepartum diet did not decrease feed intake severely, but decreased the risk of hypocalcemia (ionized Ca < 4 mg/dl) in Holstein cows. The effect of ammonium salts was even better when Ca intake was also high. Ender and Dishington (1970) also suggested that acidogenic diets reduced incidence of PP more effectively when dietary Ca also was high. These observations lead to the suggestion that diets for cows in late gestation should be acidogenic and relatively high in Ca for PP prevention.

Dairy herds with high incidence of PP often have been associated with subclinical hypomagnesemia (Allen and Sansom, 1983). Magnesium deficient cows are less capable of maintaining normal plasma Ca concentration when infused with EDTA solution (Contreras et al., 1982). The rate of Ca mobilization at parturition was lower when cows were fed a prepartum diet deficient in Mg (17 g Mg/d) (Van de Braak et al., 1987). High dietary content of Ca reduces Mg absorption but increases Mg excretion (Alcock and MacIntyre, 1964; Verdaris and Evans, 1974). Acidosis usually increases urinary excretion of Mg in other species (Nichols and Tannen, 1987),

and in ruminants (Horst and Jorgensen, 1973; Fredeen et al., 1988a). In another study we found that urinary Mg excretion tended to be lower for cows fed ammonium salts (Chapter 4). The objective of this experiment was to study the effects of dietary Mg intake on acid-base status, calcium metabolism and responses to EDTA infusion in cows fed acidogenic diets with high Ca content.

Materials and Methods

Eight nonlactating, nonpregnant Holstein cows (age 48 ± 25 mo) were assigned randomly to treatment sequences in a switchback design with three periods of 28 d each. The cows were blocked into two groups by initial BW. Cows of block 1 averaged 527±28 kg (mean ± SD) and cows of block 2 averaged 577±61 kg at the beginning of the experiment. The basal diet consisted of corn silage, ground corn and soybean meal with a silage to concentrate ration of 68:32 on DM basis (Table 5-1). The normal Mg diet was the same as the basal diet except NH_4Cl and $(NH_4)_2SO_4$ were added to the concentrate to provide 126 g of each/cow/d. In the high Mg diet, $MgSO_4$ replaced $(NH_4)_2SO_4$ to provide equivalent amount of sulfate supplied by $(NH_4)_2SO_4$ to the normal Mg diet. Urea also was added to the high Mg diet so that the two diets were isonitrogenous. All eight cows were kept in individual tie stalls in a flat barn with free access to drinking water. Cows were allowed to exercise for

Table 5-1. Ingredient composition of acidogenic diets with varying magnesium content.

Ingredients	Magnesium content	
	Normal	High
	-------(% DM)--------	
Corn silage	68.0	68.0
Ground corn	25.9	24.7
Soybean meal, 48% CP	2.2	2.2
Trace mineral salt[1]	.30	.30
Ammonium sulfate	.90	...
Ammonium chloride	.90	.90
Magnesium sulfate	...	1.68
Urea	...	.43
Calcium carbonate	1.80	1.80
Vitamin A, IU/kg	3960	3960
Vitamin D, IU/kg	1188	1188
Vitamin E, IU/kg	22	22

[1]Composed of 95 to 99% NaCl, .35% Zn, .34% Fe, .20% Mn, .033% Cu, .007% I, and .005% Co.

4 h in an open lot twice weekly. They were fed rations in two equal amounts at 0800 and 1400 h daily. During a 14 d preliminary period, the cows were fed the basal diet. The daily feed intake was adjusted to the amount (14 kg DM/d) that all cows would consume and kept the same throughout the experiment.

Samples of concentrates were taken weekly and pooled within period. Samples of corn silage also were taken weekly and dried at 60^0C for 72 h. The DM content was used to adjust the concentrate-to-corn silage ratios weekly. Daily individual water intake was measured by in-line flow meters. Cows were weighed on a digital electronic scale at the beginning and end of each period. Before the 0700 h feeding on d 25, 26 and 27 of each period, urine samples (500 ml) were collected by vulva stimulation. Urine pH was determined immediately. One sample with 1 ml concentrated HCl added to 9 ml urine and one without acid were stored frozen (-10^0C) in plastic test tubes until analyzed. One blood sample was taken anaerobically from the jugular vein and analyzed immediately for blood ionized Ca (iCa) and pH. Another 25-ml blood was taken into two 14-ml plastic tubes coated with ammonium heparin (20 Units/ml). After centrifugation at 2800 X g for 20 min, plasma was harvested and stored in small plastic tubes until analyzed for creatinine, Ca, Mg and P.

On the afternoon of d 27 of each period, catheters were inserted into both jugular veins and kept patent with saline

solution containing 100 U/ml ammonium heparin. On d 28, a 10% saline (.99% NaCl) solution of Na_2EDTA (pH adjusted to 7.4 with NaOH) was infused simultaneously into four cows within one group at an average rate of 5.4 ml/min (5.4 mg/min or .59 mg/h/kg BW). Blood samples were taken immediately before the start of the infusion and at 30 min intervals for 14.5 h after the start of the infusion. At each sampling point, one blood sample was taken anaerobically and analyzed immediately for iCa and pH. Another 25-ml blood was taken into 30-ml syringes and transferred to two 14-ml plastic tubes coated with ammonium heparin (20 U/ml). After centrifugation at 2800 X g for 20 min, plasma was harvested and stored in small plastic tubes until analyzed.

The endpoint of infusion was determined in the same way as described in Chapter 4. When one of the four cows in an infusion session showed signs of severe hypocalcemia (inability to stand), blood samples were taken simultaneously from all four cows. The infusion continued for each cow of the remaining three cows until she showed inability to stand and other signs of severe hypocalcemia, such as excessive salivation, teeth-grinding and trembling.

Feed samples were ground through 2-mm screen (Thomas-Wiley Mill, Model 4, Thomas Scientific, Philadelphia, PA). Feed DM was determined by drying at 100^0C for 24 h. Feed samples were ashed at 550^0C for 6 h. After dissolving the ash in 3 N HCl, Ca, Mg, Na, and K were determined by atomic

absorption spectrophotometry (Model 5000, Perkin Elmer, Inc., Norwalk, CT). Phosphorus was determined by colorimetry (Fiske and Subbarow, 1925). Dietary Cl was determined by potentiometric titration (Haake Buchler Instruments, Inc., Saddlebrook, NJ.). For plasma and urine samples, the same methods used for feed samples were used to determine concentrations of total Ca, Mg, P after the samples were deproteinized with 10% TCA. Concentrations of plasma and urine creatinine were determined according to Henry et al. (1974). Blood iCa and pH also were determined on a NOVA 8 Analyzer (NOVA Biochemicals, Waltham, MA). Urine pH was determined daily on subsamples by a pH meter. Titratable acidity and ammonium concentrations were determined by titration of non-acidified urine samples (Chan, 1972; Lin and Chan, 1973). Net acid concentration was the sum of titratable acidity and ammonium. Concentrations of minerals and creatinine in plasma and urine were used to calculate fractional renal excretion of minerals ([urine mineral/plasma mineral] X [plasma creatinine/urine creatinine] X 100) (Lane and Merritt, 1983).

Statistical analyses were performed by method of least squares ANOVA, using general linear mdodel procedures of SAS (Barr et al., 1982). For dependent variables with one observation in each cow-period cell, block, cow within block, period, treatment, period by block interaction, treatment by block interaction were in the mathematical model. For

variables with observations on three separate days within each cow-period, day, and interactions of day with all other terms were included in the model. Blood concentrations of iCa, plasma total Ca and Mg at different times after the start of EDTA infusion were estimated by polynomial regressions up to the fifth order, considering time as a continuous independent variable. Tests for heterogeneity of regression were performed to examine the effects of treatments over time after the start of EDTA infusion (Snedecor and Cochran, 1980).

Results

Table 5-2 shows the chemical composition of the experimental diets. Magnesium content of the high Mg diet was nearly double that of the low Mg diet. There were minor differences between the two diets in their contents of other nutrients. The dietary cation-anion difference (DCAD) calculated as milliequivalents of [(Na + K) - (CL + S)]/kg diet DM was negative for both groups, but was slightly lower than formulated in the high Mg diet. Average water intake was 26 L/cow/d with no differences between the two groups. Body weight increased by 13 kg/cow during each period similarly for both groups.

Blood pH for both groups was below 7.40, but cows fed the high Mg diet tended to have a slightly higher pH ($P < .13$) (Table 5-3). Urine pH was slightly above 6.00 with no

Table 5-2. Laboratory analysis of chemical composition (dry basis) of acidogenic diets with varying magnesium content.

Item	Magnesium content	
	Normal	High
DM, %	47.1	47.1
NE_l, Mcal/kg[1]	1.6	1.6
CP, %	13.5	13.2
ADF, %	20.5	20.4
Ca, %	1.21	1.15
P, %	.24	.24
Mg, %	.20	.37
Na, meq/kg	62	49
K, meq/kg	251	248
Cl, meq/kg	314	297
S, meq/kg	301	289
(Na+K)-(Cl+S), meq/kg	-302	-289

[1]Calculated from values of individual feedstuffs listed by NRC (1989).

Table 5-3. Effects of magnesium intake on acid-base status of Holstein cows fed acidogenic diets.

Item	Magnesium content Normal	High	SEM	P
Blood pH	7.38	7.40	.01	<.13
Urine				
pH	6.06	6.14	.05	<.26
TA[1], meq/L	4.01	3.88	.55	<.50
NAC[2], meq/L	94.28	63.25	5.23	<.01
NH_4, meq/L	90.50	59.42	4.89	<.01
TA/Cr[3]	.54	.47	.08	<.55
NH_4/Cr[3]	13.80	6.80	1.44	<.03
NAC/Cr[3]	13.29	6.33	1.39	<.02

[1]Titratable acidity equals the amount of NaOH required to titrate urine to pH 7.4.

[2]Net acid concentration equals titratable acidity plus ammonium.

[3]TA/creatinine ratio, ammonium/creatinine ratio and net acid concentration/creatinine ratio, mmol/mmol.

differences between the high Mg group and the normal Mg group. Urinary titratable acidity was similar for both the high Mg and normal Mg groups, but urine net acid concentrations and ammonium were lower for the high Mg group than for the low Mg group ($P < .01$) (Table 5-3). Blood pH was lower during the infusion and the recovery period for cows fed the normal Mg diet than for cows fed the high Mg diet (Figure 5-1)

Blood iCa was not affected by dietary treatments. Cows fed the high Mg diet tended to have a higher plasma concentration of total Ca ($P < .09$) and lower plasma concentration of P ($P< .05$) than those fed the normal Mg diet. The plasma Mg concentration was similar for both groups (Table 5-4). Urine Ca excretion was lower for the high Mg group, as shown by a lower Ca/creatinine ratio ($P < .08$) and a lower fractional excretion of Ca by the kidney ($P < .06$). Cows on the high Mg diet tended to excrete more Mg into urine, as indicated by slightly higher Mg/creatinine ratio ($P < .18$) and higher fractional excretion of Mg by the kidney ($P < .14$) (Table 5-5). There was no difference in P excretion between the two groups.

The rate of Na_2EDTA infusion averaged 5.42 ml/min. The infusion time and total volume of EDTA solution required to induce severe hypocalcemia were not different between the two treatment groups (Table 5-6). After an equal volume of EDTA solution was infused (when the first cow showed inability to

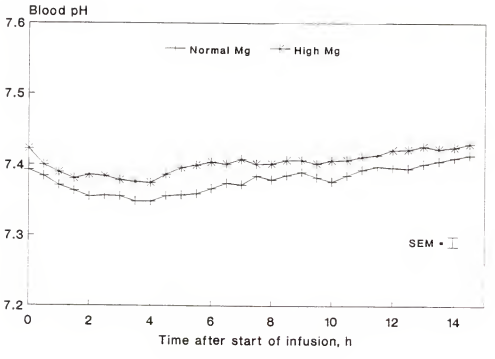

Figure 5-1. Least squares means of blood pH of Holstein cows at different sampling times. The infusion of EDTA started at 0 h and ceased at approximately 3.0 h.

Table 5-4. Effects of magnesium intake on concentrations of blood ionized calcium and plasma total calcium, magnesium and phosphorus.

Item	Magnesium content		SEM	P
	Normal	High		
Blood iCa, mg/dl	4.96	4.92	.04	<.68
Plasma				
Ca, mg/dl	9.60	9.76	.04	<.09
Mg, mg/dl	2.26	2.21	.05	<.34
P, mg/dl	5.82	5.05	.40	<.05

Table 5-5. Effects of magnesium intake on urinary excretion of calcium, magnesium and phosphorus of Holstein cows fed acidogenic diets.

Item	Magnesium content		SEM	P
	Normal	High		
Ca/Cr[1]	2.58	1.21	.41	<.08
Mg/Cr[1]	2.04	2.78	.40	<.18
P/Cr[1]	.14	.11	.03	<.50
Ca FE[2], %	8.57	3.36	1.48	<.06
Mg FE[2], %	16.61	23.81	3.15	<.14
P FE[2], %	.55	.52	.12	<.82

[1]Ca/creatinine ratio, Mg/creatinine ratio and P/creatinine ratio, mmol/mmol.

[2]Fractional excretion of Ca = [(urine Ca/plasma Ca) X (plasma creatinine/urine creatinine)] X 100. Similar calculation for fractional excretion of Mg and P.

Table 5-6. Parameters of EDTA infusion and changes in blood concentrations of ionized calcium and pH of Holstein cows.

Item	Magnesium content		SEM	P
	Normal	High		
Volume, ml	995	963	20.5	<.31
Time, min	194	190	13.7	<.42
Rate, ml/min	5.43	5.41	.02	<.49
	---After equal amount of EDTA infused---			
Blood iCa, mg/dl	2.72	2.64	.08	<.54
pH	7.36	7.38	.01	<.08

stand), blood pH but not iCa, was higher for the high Mg group ($P < .08$) (Table 5-6).

The blood iCa concentration and the plasma total Ca concentration decreased drastically when EDTA solution was infused, and then recovered gradually after the infusion stopped (Figure 5-2). Blood concentrations of ionized Ca and plasma concentrations of total Ca at different sampling times were estimated by polynomial regressions to the fifth order with sampling time as a continuous independent variable. Test of heterogeneity of regression showed no evidence that the curves for the response of blood iCa concentration to EDTA infusion for the two treatment groups were not parallel for ($P > .20$). Results were similar for plasma total Ca ($P > .20$). Figures 5-2 and 5-3 show, respectively, the least squares means of concentrations of blood iCa and plasma total Ca over times after the start of EDTA infusion.

Plasma concentrations of Mg at different times after start of the EDTA infusion were estimated by polynomial regressions to the fourth order. Test of heterogeneity of regression showed no evidence that the curves for the high Mg group and the normal Mg group were not parallel ($P > .20$). Figure 5-4 shows the least squares means of plasma Mg concentrations over time after the start of EDTA infusion.

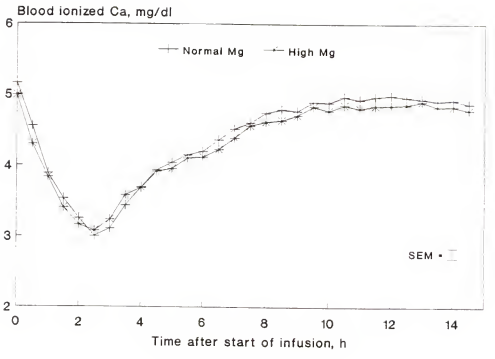

Figure 5-2. Least squares means of blood ionized Ca concentrations of Holstein cows at different sampling times. The infusion of EDTA started at 0 h and ceased at approximately 3.0 h.

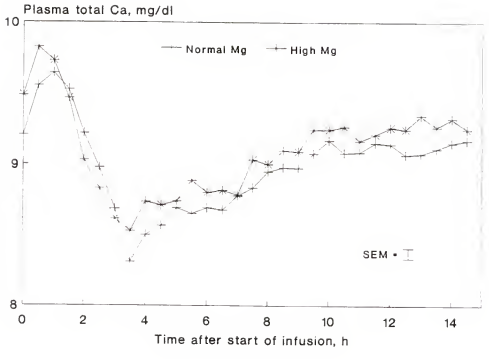

Figure 5-3. Least squares means of plasma total Ca concentrations of Holstein cows at different sampling times. The infusion of EDTA started at 0 h and ceased at approximately 3.0 h.

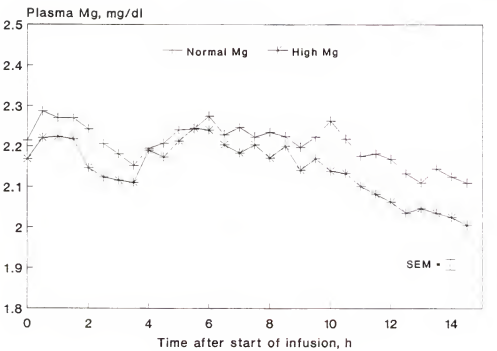

Figure 5-4. Least squares means of plasma Mg concentrations of Holstein cows at different sampling times. The infusion of EDTA started at 0 h and ceased at approximately 3.0 h.

Discussion

In the present experiment, cows were fed a fixed amount of ration throughout the experiment. Thus, the effects of feed intake were nonconsequential. The effects due to cow variation could be accounted for statistically to some extent because of the switchback design used. As described in Chapter 4, the effects of parturition and health and management factors associated with parturition did not exist in this study because the cows were nonlactating and nonpregnant. The metabolic effects of Mg intake might be estimated more precisely than in a continuous trial with late gestation cows.

Several previous studies including one of our own (Chapter 4) showed that acidogenic salts caused acidosis and increased cows' ability to maintain blood iCa concentration (Kendall et al., 1969; Vagg and Payne, 1970; Block, 1984; Oetzel et al., 1988). In the present study, both treatment diets were acidogenic. This was reflected by relatively low blood pH (pH < 7.40) and acidic urine (pH < 7.00) and positive acid excretion by both treatment groups. The two diets were isonitrogenous except the high Mg diet had urea as a nitrogen source to match the nitrogen from $(NH_4)_2SO_4$ added to the normal Mg diet. The higher urine excretion of ammonium by cows fed the normal Mg diet was an indication of increased acid excretion (Table 5-3). Cows on the normal Mg diet tended to

have lower blood pH and higher ammonium excretion, indicating the normal Mg diet was more acidogenic than the high Mg diet. The slight difference in DCAB between the two diets may not be solely responsible. The difference in acidogenicity between $(NH_4)_2SO_4$ and $MgSO_4$ likely contributed to the difference in acid-base balance between the two groups.

The blood concentration of iCa for both groups in this study was similar to that of the group fed ammonium salts, but higher than that of the control group in the other experiment (Chapter 4), because cows on both high Mg and normal Mg groups were acidemic. There was only minor difference between the high Mg and normal Mg groups in total plasma Ca, but no difference in iCa concentrations (Table 5-4). Concentrations of plasma Mg were relatively constant and not affected even by a diet deficient in Mg except at parturition (Van de Braak et al., 1987). In the present study, the plasma concentration of Mg was similar for both groups. Others showed that diets deficient in Mg caused a decrease in plasma Mg concentration (Contreras et al., 1982). In our study, increasing Mg intake above the requirement (NRC, 1989) did not affect plasma Mg concentration. Plasma P was slightly lower for the high Mg group than for the normal Mg group (Table 5-4). High Mg intake might reduce P absorption (Care et al., 1980).

Urinary excretion of Ca and Mg are interrelated. Increased excretion of Ca was accompanied by increased excretion of Mg and vice versa in the dog, cat and rabbit

(Mendel and Benedict, 1909a, 1909b). In goats, dietary induced acidosis also increased Ca excretion accompanied by increased Mg excretion (Fredeen et al., 1988a). There may be competition between the two elements for a common transporting system both in the intestine and in the renal tubules (Alcock and MacIntyre, 1962). However, the association between hypercalciuria and hypermagnesemia might be disrupted by acidosis (Stacy and Wilson, 1970) or Mg status. In the present study, Mg excretion tended to increase for the high Mg group, as shown by a slightly higher Mg/creatinine ratio ($P < .18$) and higher fractional excretion of Mg ($P < .14$) (Table 5-5). However, Ca excretion decreased as shown by a lower Ca/creatinine ratio ($P < .08$) and lower fractional excretion of Ca ($P < .06$) for the high Mg group compared with the normal Mg group. In another study (Chapter 4), increased Ca excretion was accompanied by a lower excretion of Mg in cows fed ammonium salts. The complex interaction of Mg, Ca and acidosis at the intestine and in the renal tubule may be responsible for such differences between our study and others.

Previous studies showed that Mg deficiency reduced Ca mobilization from bones (Contreras et al., 1982; Van de Braak et al., 1986). In the present study, the amount of EDTA solution and time of infusion required to cause severe hypocalcemia (inability to stand) were not different between treatments. The blood iCa concentration decreased and recovered at similar rate for both groups after the infusion

stopped, as suggested by the parallel curves for blood iCa and plasma total Ca over sampling times (Figures 5-2 and 5-3). These results suggest that dietary Mg intake did not affect the ability of cows to maintain normal blood Ca concentration under the conditions of this study. Only Mg deficiency would impair the release of parathyroid hormone and response of bone and intestine to parathyroid and $1,25\text{-}(OH)_2D_3$ (MacManus et al., 1971). In this study plasma Mg concentration was within the normal range, indicating Mg was not deficient. Therefore, supplementation of Mg was not necessary at the rate of Ca intake in this study.

The design of the study also permited a relative comparison between $MgSO_4$ and $(NH_4)_2SO_4$, assuming urea had no effects on acid-base balance. It appears that $MgSO_4$ was less effective than $(NH_4)_2SO_4$ as an acidogenic agent, as suggested by higher blood pH and lower urinary ammonium and net acid concentrations (Table 5-3). However, no differences between the two groups could be detected in the response to EDTA infusion, indicating that $MgSO_4$ had similar effect on bone Ca mobilization as $(NH_4)_2SO_4$.

In conclusion, increased Mg intake decreased urine Ca excretion but had little effect on the response to EDTA infusion in cows fed acidogenic diets with relatively high Ca content. Magnesium sulfate was slightly less acidogenic than ammonium sulfate.

CHAPTER 6
EFFECTS OF PREPARTAL DIETARY CATION-ANION DIFFERENCE ON CALCIUM METABOLISM, HEALTH AND PRODUCTION PERFORMANCE OF HOLSTEIN DAIRY COWS

Introduction

The period around parturition is a particularly critical time in the productive cycle of dairy cows because of the tremendously dynamic physiological changes during this period. Prevention of metabolic diseases such as parturient paresis (PP) will impact positively on the health and subsequent lactational and reproductive performance. Researchers in Norway first showed PP was induced by alkaline diets but prevented by silage preserved with mineral acids (HCl and H_2SO_4), regardless of Ca and P intake (Ender et al., 1962). They later found that diets with negative dietary cation-anion difference (-DCAD) (with supplemental $CaCl_2$, $MgSO_4$ and $(Al)_2(SO_4)_3$) also reduced PP (Dishington, 1975). However, this dietary manipulation was not used extensively in Scandinavian countries because the traditional feeding practices for dry cows involved grazing or preserved forage with a small amount of concentrate fed separately. With these practices, unpalatability of the anionic salts could be a problem (personal communication, D. K. Beede). However, Block (1984) in Canada showed that the mixture used by Norwegian workers did not present problems with feed intake when mixed

in a total mixed ration. Incidence of PP for cows fed a control diet was 47%, but 0% for cows fed diets with negative DCAD. Additionally, cows fed the diet with -DCAD prepartum produced 7% more milk than the control group managed identically postpartum. Curtis et al. (1984) reported that PP was associated closely with other periparturient disorders including dystocia, retained placenta, ketosis, displaced abomasum and mastitis. Prevention of PP and hypocalcemia may reduce or eliminate some of these problems, indirectly improving postpartum health and production performance.

Oetzel et al. (1988) showed that diets made negative in DCAD with NH_4Cl and $(NH_4)_2SO_4$ reduced the risk of hypocalcemia significantly, although the effect on PP incidence was not significant. Our own study (Chapter 4) also showed that ammonium salts enhanced Ca metabolism and increased cows' ability to maintain normal blood concentrations of ionized Ca (iCa) when the demand for Ca was increased suddenly (Chapter 4). Magnesium sulfate was slightly less acidogenic than the ammonium salts, but had similar effects on the response to EDTA infusion as the ammonium salts (Chapter 5). Diets with -DCAD appeared to be more effective when dietary Ca content was relatively high (Ender and Dishington, 1970; Oetzel et al., 1988). The objective of the present study was to evaluate the effects of a prepartal diet with -DCAD and high Ca content on Ca metabolism, incidence of PP and other metabolic disorders, and lactational performance in a large commercial dairy herd.

MATERIALS AND METHODS

A total of 224 first parity and 286 later parity animals expected to calve from January through April 30, 1990 were assigned to two treatment groups by ear tag number. Those with an even last digit were fed a control diet with a positive dietary cation-anion difference (+DCAD), and those with an odd last digit on their ear tag were fed a treatment diet with a negative DCAD (-DCAD). Approximately 3 wk before expected calving date, the animals were transferred to two adjacent lots and group-fed their assigned diets. The formulation of the two diets are presented in Table 6-1. The +DCAD diet was formulated to meet the NRC (1989) recommendations for dry pregnant cows. It was similar in basic composition to the -DCAD diet except it contained fermented ammoniated whey, but no acidogenic salts (NH_4Cl, $(NH_4)_2SO_4$ and $MgSO_4$). The average DM intake was estimated to be 10.3 kg/cow/d. The -DCAD diet was supplemented with NH_4Cl (108 g/cow/d), $(NH_4)_2SO_4$ (53 g/cow/d) and $MgSO_4$ (34 g/cow/d), but it contained no fermented ammoniated whey so that the two diets had similar concentrations of non-protein nitrogen. All animals also had ad libitum access to bermudagrass hay rolls and grazing in the lots. Samples of total mixed rations were taken biweekly and sent to DHIA Forage Laboratory (Ithaca, NY) for nutrient analysis.

Table 6-1. Formulated ingredient composition of diets with varying dietary cation-anion difference (DCAD).

Ingredient	+DCAD	-DCAD
	------- % of DM -------	
Corn silage	24.07	23.74
Bermudagrass silage	20.04	19.77
Wet brewers grains	8.44	8.33
Hominy	30.06	29.65
Bermudagrass hay	12.18	12.02
Fermented ammoniated whey	4.12	...
+DCAD premix	1.08	...
-DCAD premix	...	6.46
NH_4Cl, g/d	...	108
$(NH_4)_2SO_4$, g/d	...	53
$MgSO_4$[1], g/d	...	34

[1]Hydrated form: $MgSO_4.7H_2O$, epsom salts.

Body condition scores and edema scores of the animals before parturition were recorded by a veterinarian weekly. Body condition was scored from 1 to 5 (Braun et al., 1987). Edema scores ranged from 0 to 3, where 0 = no edema, 1 = mild edema of the umbilical area with no udder edema; 2 = moderate edema of the umbilical area with mild edema of the udder; 3 = moderate to severe edema of the umbilical area with moderate to severe edema of the udder.

All animals were examined for any health problems within 12 h post calving by personnel in the hospital barn on the dairy and confirmed by veterinarians. A venous blood sample was taken from each cow within 12 h postpartum and before any treatment for PP. The samples were taken to the laboratory, centrifuged (2000 X g) and serum was harvested. Serum was analyzed for iCa and pH immediately (NOVA 8 Analyzer, NOVA Biochemicals, Waltham, MA). A duplicate serum sample was stored frozen (-10^0C) until analyzed for serum electrolytes and other metabolites. Parturient paresis was diagnosed when cows showed staggering or difficulty in standing, and was confirmed by serum iCa concentrations of < 3.0 mg/dl prior to therapeutic intervention. Cows were defined as hypocalcemic if the serum concentration of iCa was < 4 mg/dl.

After calving all cows entered the lactation herd and were fed and managed the same way, regardless of prepartum treatment. Production data were collected monthly by DHIA recording system (Raleigh, NC).

Serum Na, K, Cl, and total bicarbonate were determined on a 664 Fast 4 System (CIBA-CORNING Diagnostics Corp., Medfield, MA). Serum concentrations of Ca, P, Mg, total protein, albumin, globulin, glucose, serum urea nitrogen, creatinine, total bilirubin, activities of alkaline phosphatase, serum glutamic-oxalocetic tramsaminase (SGOT), and gamma-glutamyl-transferase (SGGT) were determined on 550 Express (CIBA-CORNING Diagnostics Corp., Medfield, MA).

Statistical analyses were conducted by method of least squares ANOVA, using general linear model procedures of SAS (Barr et al., 1982). Serum iCa and other chemical components were analyzed with month of calving, treatment, parity and their interactions in the mathematical model. The same model was used to analyze the incidence of diseases. Incidence of a disease was coded as 0 if a cow did not have that disease or 1 if she had the disease. Because of variation in expected calving date, the duration (number of days) each cow was fed the experimental diets varied (overall, 24.5 ± 1 d). The duration that each cow was fed the treatment diet was used as an continuous independent covariate in the model. Total milk production of the initial 7 mo of the experiment and predicted 305-d milk production were analyzed with month of calving, treatment, parity and their interactions in the model with duration and days in milk as continuous independent variables. Milk production measured on monthly test days was analyzed with month of calving, treatment, parity and their

interactions with days in milk as a continuous independent variable. The effect of cow was estimated and used to test the effects of month of calving, treatment and parity.

RESULTS

Laboratory analysis of the diets is presented in Table 6-2. The concentrations of Ca in the diets were higher than intended by formulation because the Ca concentration of forages was higher than originally estimated. The Ca concentration of the +DCAD diet was lower than that of the -DCAD diet as intended.

The experiment included a total of 224 first parity and 286 later parity animals. Table 6-3 shows the distribution of animals and the number of days experimental diets were fed to the different groups. Body condition scores averaged 3.35 (SE = .03) with no difference between the two treatments (Table 6-4). Edema scores was similar for both treatment groups (.34 for +DCAD group and .36 for -DCAD group (SE = .07)). Body condition scores increased from 3.19 to 3.54 ($P < .01$), but edema scores decreased from 1.11 to .16 ($P < .01$) as parity increased from one to five (Table 6-4).

Serum concentrations of Na and K were similar for both treatment groups, but Cl concentrations were higher for the -DCAD group than for the +DCAD group ($P < .01$) (Table 6-5a). Serum concentrations of iCa, total Ca and P, but not Mg, were higher for the -DCAD group than for the +DCAD group (Table 6-

Table 6-2. Laboratory analysis of chemical composition (dry basis) of diets varying in dietary cation-anion difference (DCAD)[1].

Item	+DCAD	-DCAD
DM, %	36.37	38.94
CP, %	14.75	13.54
ADF, %	29.60	26.67
NE_l[2], Mcal/kg	1.45	1.50
Ca, %	.92	1.81
P, %	.34	.29
Mg, %	.24	.33
Na, meq/kg	72.57	53.87
K, meq/kg	319.36	272.15
Cl, meq/kg	154.01	299.57
S, meq/kg	187.92	273.70
Fe, ppm	351	376
Zn, ppm	59	120
Cu, ppm	22	30
Mn, ppm	102	176
Mo, ppm	1.8	1.8
(Na + K) - (Cl + S), meq/kg	50.00	-247.25

[1]From biweekly bunk samples of each dietary treatment.

[2]Calculated from values of individual feedstuffs listed by NRC (1990).

Table 6-3. Distribution of experimental animals and the duration (days fed experimental diets) in treatment groups with different parity (L) and month of calving (M).

L	No. of Cows				Duration		
	+DCAD	-DCAD	Total		+DCAD	-DCAD	Mean
1	112	112	224		27±1	28±1	28±1
2	53	58	111		24±2	21±1	23±1
3	36	38	74		26±2	25±2	25±1
4	31	33	64		21±2	26±2	23±1
5	18	19	37		24±2	22±3	23±2
Total	250	260	510				
M							
Jan	86	90	176		24±1	24±1	24±1
Feb	57	67	124		24±2	24±2	24±1
Mar	50	52	102		26±2	23±2	25±1
Apr	57	51	108		25±2	25±2	25±1
Total	250	260	510	Mean	25±1	24±1	

Table 6-4. Body condition score and edema score of animals in treatment groups with different parity (L) and month of calving (M).

L	Condition score[1]					Edema score[2]				
	+DCAD	-DCAD	Mean	SE	P	+DCAD	-DCAD	Mean	SE	P
1	3.21	3.18	3.19			1.09	1.14	1.11		
2	3.31	3.15	3.23			.26	.20	.23		
3	3.32	3.40	3.36			.09	.10	.10		
4	3.36	3.44	3.40			.17	.06	.12		
5	3.53	3.55	3.54			.06	.27	.16		
SE			.05					.10		
P			<.01					<.01		
M										
Jan	3.30	3.39	3.35			.32	.25	.29		
Feb	3.56	3.51	3.53			.37	.33	.35		
Mar	3.24	3.32	3.28			.37	.53	.45		
Apr	3.28	3.15	3.22			.27	.31	.29		
SE			.03					.10		
P			<.01					<.60		
Mean	3.35	3.35		.03	.96	.34	.36		.07	<.85

[1]Body condition was scored from 1 to 5 (Braun et al., 1987).

[2]Edema scores ranged from 0 to 3, where 0 = no edema, 1 = mild edema of the umbilical area with no udder edema; 2 = moderate edema of the umbilical area with mild edema of the udder; 3 = moderate to severe edema of the umbilical area with moderate to severe edema of the udder.

Table 6-5a. Effects of prepartal dietary cation-anion difference (DCAD) on serum concentrations of macrominerals, and serum pH, HCO_3 and anion gap.

DCAD	Na meq/L	K meq/L	Cl meq/L	iCa mg/dl	Ca mg/dl	P mg/dl	Mg mg/dl	pH	HCO_3 meq/L	Anion gap[1] meq/L
+	144.9	4.45	106.4	3.80	7.05	3.64	2.07	7.44	24.8	18.0
-	144.9	4.54	108.4	4.31	7.94	4.44	2.02	7.43	22.6	18.3
SE	.4	.05	.4	.03	.10	.11	.08	.01	.2	.3
P	<.95	<.22	<.01	<.01	<.01	<.01	<.67	<.98	<.01	<.37

[1]Anion gap = the serum concentration of (Na meq/L + K meq/L) - (Cl meq/L + HCO_3 meq/L).

5a). Serum pH and anion gap were similar but concentrations of total bicarbonate (HCO_3) were lower for the -DCAD group ($P < .01$). Table 6-5b shows the effect of month of calving on serum concentrations of macrominerals, and pH, HCO_3 and anion gap. No clear time trends were observed for Na and K, but there was an overall month effect on serum K concentrations ($P < .01$). The concentrations of Cl ($P < .09$) and anion gap ($P < .05$) decreased as the month of calving progressed from January to April. The month of calving also had effects on serum concentrations of iCa ($P < .01$), Ca ($P < .01$), P ($P < .03$), Mg ($P < .01$), HCO_3 ($P < .01$), and serum pH ($P < .01$). There was no interaction between the month of calving and dietary treatment on the serum concentrations of most macrominerals except Na and Cl (Table 6-5c). There was an interaction of month of calving and dietary treatment on anion gap ($P < .07$). Serum concentrations of Na, K and Mg were not affected by parity (Table 6-5d). Concentrations of iCa, Ca, P and HCO_3 decreased, but anion gap increased as parity increased. The concentration of Cl was highest for the third parity. The concentrations of Na, K, Mg, HCO_3, anion gap, and pH were not affected by the interaction between dietary treatment and parity (Table 6-5e). The concentration of Cl increased in +DCAD group as parity increased up through the fifth parity, but increased through the third parity and

Table 6-5b. Effects of the month of calving (M) on serum concentrations of macrominerals, and serum pH, HCO_3 and anion gap.

M	Na meq/L	K meq/L	Cl meq/L	iCa mg/dl	Ca mg/dl	P mg/dl	Mg mg/dl	pH	HCO_3 meq/L	Anion gap[1] meq/L
Jan	145.5	4.69	108.4	3.92	7.47	3.92	1.83	7.53	23.2	18.5
Feb	144.7	4.33	107.4	3.94	6.97	3.73	1.81	7.40	22.7	18.9
Mar	144.3	4.50	107.0	4.11	7.61	4.12	2.20	7.43	23.9	17.8
Apr	145.1	4.46	106.9	4.26	7.93	4.40	2.33	7.38	25.1	17.5
SE	.6	.07	.5	.05	.15	.15	.11	.02	.3	.4
P	<.45	<.01	<.09	<.01	<.01	<.03	<.01	<.01	<.01	<.05

[1]Anion gap = the serum concentration of (Na meq/L + K meq/L) - (Cl meq/L + HCO_3 meq/L).

Table 6-5c. Effects of interaction between the month of calving (M) and prepartal dietary cation-anion difference (DCAD) on serum concentrations of macrominerals, and serum pH, HCO_3 and anion gap.

DCAD	M	Na meq/L	K meq/L	Cl meq/L	iCa mg/dl	Ca mg/dl	P mg/dl	Mg mg/dl	pH	HCO_3 meq/L	Anion gap[1] meq/L
+	Jan	146.8	4.66	108.4	3.65	6.94	3.42	1.92	7.55	24.4	18.6
+	Feb	144.7	4.33	106.9	4.19	6.64	3.34	1.79	7.51	23.7	18.4
+	Mar	143.9	4.37	105.8	3.68	7.23	3.59	2.19	7.38	24.7	17.6
+	Apr	144.1	4.43	104.7	4.19	7.39	4.22	2.37	7.43	26.4	17.3
-	Jan	144.2	4.71	108.5	3.91	8.01	4.42	1.74	7.44	21.9	18.4
-	Feb	144.6	4.33	107.9	4.32	7.30	4.12	1.83	7.41	21.7	19.3
-	Mar	144.7	4.62	108.2	3.96	7.99	4.65	2.22	7.36	23.1	17.9
-	Apr	146.2	4.48	109.1	4.55	8.47	4.58	2.29	7.39	23.8	17.7
SE		.8	.10	.7	.07	.18	.21	.15	.02	.4	.4
P		<.02	<.65	<.03	<.65	<.59	<.42	<.83	<.64	<.73	<.07

[1]Anion gap = the serum concentration of (Na meq/L + K meq/L) - (Cl meq/L + HCO_3 meq/L).

Table 6-5d. Effects of parity (L) on serum concentrations of macrominerals, and serum pH, HCO_3 and anion gap.

L	Na meq/L	K meq/L	Cl meq/L	iCa mg/dl	Ca mg/dl	P mg/dl	Mg mg/dl	pH	HCO_3 meq/L	Anion gap[1] meq/L
1	144.2	4.36	106.4	4.60	8.54	4.84	1.96	7.45	24.5	17.5
2	144.6	4.49	107.5	4.32	8.00	4.43	1.99	7.42	23.8	17.8
3	145.3	4.48	108.5	4.10	7.72	4.14	1.98	7.46	23.6	17.6
4	145.4	4.56	107.2	3.77	6.95	3.58	2.06	7.42	23.9	18.7
5	145.0	4.58	107.5	3.48	6.27	3.23	2.22	7.43	22.8	19.2
SE	.6	.07	.5	.05	.14	.16	.11	.01	.3	.4
P	<.43	<.14	<.03	<.01	<.01	<.01	<.70	<.20	<.01	<.03

[1]Anion gap = the serum concentration of (Na meq/L + K meq/L) - (Cl meq/L + HCO_3 meq/L).

Table 6-5e. Effects of interaction between parity (L) and prepartal dietary cation-anion difference (DCAD) on serum concentrations of macrominerals, and serum pH, HCO_3 and anion gap.

DCAD	L	Na meq/L	K meq/L	Cl meq/L	iCa mg/dl	Ca mg/dl	P mg/dl	Mg mg/dl	pH	HCO_3 meq/L	Anion gap[1] meq/L
+	1	144.6	4.37	105.0	4.51	8.49	4.65	2.06	7.46	25.8	17.3
+	2	144.4	4.39	106.0	4.07	7.64	4.29	1.90	7.42	25.0	17.6
+	3	144.2	4.41	106.0	3.74	6.95	3.52	1.96	7.48	25.1	17.4
+	4	145.9	4.45	107.1	3.50	6.40	2.85	2.08	7.39	25.1	18.0
+	5	145.3	4.63	107.3	3.17	5.76	2.91	2.32	7.43	23.1	19.4
-	1	143.7	4.36	107.0	4.69	8.58	5.03	1.87	7.44	23.2	17.7
-	2	144.8	4.58	108.9	4.57	8.36	4.57	2.07	7.42	22.5	17.9
-	3	146.4	4.56	111.1	4.47	8.50	4.75	2.00	7.44	22.1	17.7
-	4	144.8	4.67	107.3	4.04	7.50	4.32	2.04	7.45	22.7	19.3
-	5	144.8	4.52	107.7	3.79	6.79	3.55	2.12	7.41	22.6	18.9
SE		.8	.11	.8	.07	.20	.23	.16	.02	.4	.5
P		<.28	<.49	<.02	<.01	<.01	<.02	<.61	<.28	<.44	<.80

[1]Anion gap = the serum concentration of (Na meq/L + K meq/L) - (Cl meq/L + HCO_3 meq/L).

decreased thereafter in the -DCAD group. As parity increased, the concentrations of iCa, Ca and P decreased more in the +DCAD group than in the -DCAD group (Table 6-5e).

Serum concentrations of proteins, glucose, alkaline phosphatase, glutamic-oxalocetic transaminase (SGOT) and gamma-glutamyl-transferase (SGGT) were similar for both treatment groups (Table 6-6a). Serum concentrations of total bilirubin and serum urea nitrogen (SUN) were lower, but creatinine was higher in the -DCAD group than in the +DCAD group. As the month of calving progressed from January to April, serum concentrations of total protein, albumin and globulin increased, but concentrations of glucose decreased ($P < .04$) and concentrations of creatinine varied ($P < .04$), with no major changes in albumin/globulin ratio, or concentrations of alkaline phosphatase, SGOT and SGGT (Table 6-6b). There were no interactions between treatment and the month of calving on serum concentrations of these components except for creatinine concentrations ($P < .03$) (Table 6-6c). Concentrations of serum total proteins, albumin and globulin increased with a decrease in albumin/globulin ratio as parity increased (Table 6-6d). Concentrations of total bilirubin increased, but concentrations of alkaline phophatase, SGOT and SGGT decreased as parity increased (Table 6-6d). There were no interactions between parity and dietary treatment on serum concentrations of total protein, globulin, glucose, SUN, alkaline phosphatase, SGOT and SGGT (Table 6-6e). There were

Table 6-6a. Effects of prepartal dietary cation-anion difference (DCAD) on serum concentrations of proteins and metabolites.

DCAD	Total protein g/dl	Albumin g/dl	Globulin g/dl	A/G[1]	Glucose mg/dl	Total bilirubin mg/dl	SUN[2] mg/dl	Cr[3] mg/dl	AP[4] U/L	SGOT[5] U/L	SGGT[6] U/L
+	6.10	3.58	2.51	1.46	60.8	.586	17.6	1.29	54.71	82.01	26.79
-	6.07	3.57	2.50	1.46	59.6	.462	16.4	1.34	53.30	79.20	24.52
SE	.04	.03	.03	.02	2.0	.019	.5	.02	2.01	2.53	4.04
P	<.65	<.92	<.75	<.99	<.68	<.01	<.08	<.01	<.62	<.43	<.69

[1]Albumin/globulin ratio, g/g.
[2]Serum urea nitrogen.
[3]Creatinine.
[4]Alkaline phosphatase.
[5]Serum glutamic-oxalocetic transaminase.
[6]Serum gamma-glutamyltransferase.

Table 6-6b. Effects of the month of calving (M) on serum concentrations of proteins and metabolites.

M	Total protein g/dl	Albumin g/dl	Globulin g/dl	A/G[1]	Glucose mg/dl	Total bilirubin mg/dl	SUN[2] mg/dl	Cr[3] mg/dl	AP[4] U/L	SGOT[5] U/L	SGGT[6] U/L
Jan	5.97	3.52	2.45	1.47	63.9	.482	16.6	1.31	55.12	78.64	31.59
Feb	5.96	3.52	2.45	1.47	66.3	.553	15.9	1.38	49.94	76.33	23.57
Mar	6.14	3.60	2.55	1.46	59.1	.530	16.7	1.26	54.69	86.39	23.18
Apr	6.26	3.67	2.58	1.44	51.4	.533	18.8	1.30	56.26	81.05	24.28
SE	.05	.03	.04	.04	2.7	.026	.7	.03	2.80	3.54	5.50
P	<.01	<.02	<.09	<.91	<.01	<.16	<.05	<.04	<.42	<.24	<.55

[1]Albumin/globulin ratio, g/g.
[2]Serum urea nitrogen.
[3]Creatinine.
[4]Alkaline phosphatase.
[5]Serum glutamic-oxalocetic transaminase.
[6]Serum gamma-glutamyltransferase.

Table 6-6c. Effects of interaction between the month of calving (M) and prepartal dietary cation-anion difference (DCAD) on serum concentrations of proteins and metabolites.

DCAD	M	Total protein g/dl	Albumin g/dl	Globulin g/dl	A/G[1]	Glucose mg/dl	Total bilirubin mg/dl	SUN[2] mg/dl	Cr[3] mg/dl	AP[4] U/L	SGOT[5] U/L	SGGT[6] U/L
+	Jan	6.05	3.57	2.47	1.49	66.8	.517	16.7	1.32	51.37	80.08	33.66
+	Feb	6.04	3.54	2.49	1.46	65.8	.592	16.1	1.29	51.15	80.62	23.72
+	Mar	6.12	3.58	2.54	1.46	59.5	.603	17.8	1.27	55.81	85.37	23.52
+	Apr	6.18	3.62	2.55	1.44	51.0	.633	20.0	1.27	60.50	81.97	26.27
-	Jan	5.89	3.46	2.43	1.46	61.0	.446	16.5	1.30	58.86	77.21	29.52
-	Feb	5.88	3.50	2.40	1.49	66.9	.513	15.6	1.47	48.73	72.04	23.41
-	Mar	6.17	3.63	2.55	1.47	58.7	.458	15.6	1.25	53.56	87.41	22.84
-	Apr	6.34	3.71	2.62	1.44	51.7	.432	17.7	1.33	52.02	80.13	22.29
SE		.07	.05	.06	.05	3.9	.040	1.0	.04	3.97	5.23	7.93
P		<.10	<.14	<.68	<.91	<.72	<.30	<.59	<.03	<.16	<.78	<.99

[1]Albumin/globulin ratio, g/g.
[2]Serum urea nitrogen.
[3]Creatinine.
[4]Alkaline phosphatase.
[5]Serum glutamic-oxalocetic transaminase.
[6]Serum gamma-glutamyltransferase.

Table 6-6d. Effects of parity (L) on serum concentrations of proteins and metabolites.

L	Total protein g/dl	Albumin g/dl	Globulin g/dl	A/G[1]	Glucose mg/dl	Total bilirubin mg/dl	SUN[2] mg/dl	Cr[3] mg/dl	AP[4] U/L	SGOT[5] U/L	SGGT[6] U/L
1	5.86	3.50	2.36	1.52	60.3	.386	18.0	1.26	77.02	93.73	35.85
2	5.89	3.52	2.37	1.51	55.1	.452	16.7	1.29	58.27	84.06	28.91
3	5.99	3.58	2.40	1.53	62.4	.528	17.8	1.33	48.16	77.42	21.56
4	6.23	3.61	2.61	1.41	65.3	.648	16.6	1.40	46.05	77.05	21.20
5	6.45	3.66	2.78	1.34	57.8	.607	15.8	1.29	40.51	70.76	20.76
SE	.05	.04	.05	.04	2.9	.029	.7	.03	4.04	3.83	6.10
P	<.01	<.02	<.01	<.01	<.13	<.01	<.18	<.01	<.01	<.01	<.11

[1]Albumin/globulin ratio, g/g.
[2]Serum urea nitrogen.
[3]Creatinine.
[4]Alkaline phosphatase.
[5]Serum glutamic-oxalocetic transaminase.
[6]Serum gamma-glutamyltransferase.

Table 6-6e. Effects of interaction between parity (L) and prepartal dietary cation-anion difference (DCAD) on serum concentrations of proteins and metabolites.

DCAD	L	Total protein g/dl	Albumin g/dl	Globulin g/dl	A/G[1]	Glucose mg/dl	Total bilirubin mg/dl	SUN[2] mg/dl	Cr[3] mg/dl	AP[4] U/L	SGOT[5] U/L	SGGT[6] U/L
+	1	5.89	3.54	2.36	1.53	60.0	.44	17.9	1.26	79.98	94.64	36.95
+	2	5.93	3.48	2.44	1.47	58.4	.54	18.0	1.32	57.27	81.08	32.74
+	3	5.93	3.52	2.41	1.51	60.1	.57	17.7	1.27	49.41	81.98	20.63
+	4	6.29	3.70	2.58	1.45	68.6	.74	16.6	1.32	42.29	78.81	22.42
+	5	6.45	3.65	2.78	1.35	56.7	.63	18.0	1.29	44.60	73.53	21.24
-	1	5.83	3.46	2.36	1.50	60.6	.33	18.2	1.25	74.06	92.81	34.74
-	2	5.85	3.55	2.31	1.56	51.8	.35	15.3	1.27	59.28	87.04	25.08
-	3	6.05	3.65	2.40	1.55	64.7	.48	18.0	1.38	46.90	72.87	22.49
-	4	6.17	3.53	2.65	1.37	62.1	.55	16.7	1.48	49.81	75.29	19.98
-	5	6.45	3.68	2.78	1.33	58.8	.58	13.6	1.30	36.43	67.98	20.28
SE		.08	.05	.07	.05	4.2	.04	1.0	.04	4.29	5.41	8.62
P		<.63	<.06	<.59	<.41	<.54	<.04	<.14	<.05	<.36	<.64	<.98

[1]Albumin/globulin ratio, g/g.
[2]Serum urea nitrogen.
[3]Creatinine.
[4]Alkaline phosphatase.
[5]Serum glutamic-oxalocetic transaminase.
[6]Serum gamma-glutamyltransferase.

interactions between parity and dietary treatment on albumin concentrations ($P < .06$) and creatinine concentrations ($P < .05$). Concentrations of total bilirubin increased linearly as parity increased in the -DCAD group, but peaked at the fourth parity in the +DCAD group ($P < .04$).

Incidence of hypocalcemia and PP were much lower in the -DCAD group than in the +DCAD group, but incidences of other periparturient disorders were similar for both groups (Table 6-7a). As the month of calving changed from January to April, incidence of hypocalcemia and PP decreased (Table 6-7b). Incidence of retained placenta and metritis also varied from month to month. Incidence of dystocia, displaced abomasum, ketosis and mastitis were similar for all months of calving. Incidences of PP and displaced abomasum, but not the other disorders, was affected by interaction between the month of calving and treatment (Table 6-7c). As parity increased, incidence of hypocalcemia, PP, retained placenta, ketosis and metritis all increased, but incidence of dystocia decreased (Table 6-7d). There was significant interaction between parity and treatment on the incidence of hypocalcemia, PP, displaced abomasum and ketosis, but not on other disorders (table 6-7e).

Daily milk production measured on test days was not affected by treatment (Table 6-8a) or the month of calving (table 6-8b). For cows that calved in January and March, protein yield and milk yield were higher for the -DCAD group,

Table 6-7a. Effects of prepartum dietary cation-anion difference (DCAD) on incidence of hypocalcemia (HC), parturient paresis (PP), and other peripartureint disorders[1].

DCAD	HC[2]	PP	Retained placenta	Dystocia	Displaced abomasum	Ketosis	Metritis	Mastitis
+	.501	.089	.218	.162	.039	.029	.208	.169
-	.194	.036	.206	.117	.025	.030	.189	.158
SE	.026	.012	.026	.030	.012	.011	.029	.028
P	<.01	<.01	<.76	<.29	<.40	<.92	<.64	<.80

[1]If a cow had a disorder, the incidence of the disorder was 1, whereas if the cow did not have a certain disorder, the incidence of that disorder was 0.
[2]Hypocalcemia = 1 if the serum iCa concentration was 4.00 mg or less /dl.

Table 6-7b. Effects of the month of calving (M) on incidence of hypocalcemia (HC), parturient paresis (PP), and other periparturient disorders[1].

M	HC[2]	PP	Retained placenta	Dystocia	Displaced abomasum	Ketosis	Metritis	Mastitis
Jan	.452	.071	.176	.101	.037	.027	.205	.199
Feb	.428	.102	.171	.121	.018	.024	.087	.207
Mar	.276	.058	.164	.151	.039	.054	.204	.127
Apr	.233	.020	.338	.184	.033	.014	.298	.121
SE	.035	.017	.034	.042	.016	.016	.040	.040
P	<.01	<.01	<.01	<.49	<.83	<.39	<.01	<.28

[1]If a cow had a disorder, the incidence of the disorder was 1, whereas if the cow did not have a certain disorder, the incidence of that disorder was 0.
[2]Hypocalcemia = 1 if the serum iCa concentration was 4.00 mg/dl or less.

Table 6-7c. Effects of interaction between the month of calving (M) and prepartal dietary cation-anion difference (DCAD) on incidence of hypocalcemia (HC), parturient paresis (PP), and other peripartal disorders[1].

DCAD	M	HC[2]	PP	Retained placenta	Dystocia	Displaced abomasum	Ketosis	Metritis	Mastitis
+	Jan	.586	.104	.227	.100	.045	.019	.235	.198
+	Feb	.572	.164	.188	.156	.031	.048	.061	.220
+	Mar	.437	.049	.165	.112	.078	.049	.259	.137
+	Apr	.409	.040	.291	.279	.000	.000	.278	.120
-	Jan	.317	.038	.125	.101	.028	.035	.174	.201
-	Feb	.284	.040	.154	.086	.005	.000	.114	.193
-	Mar	.116	.068	.163	.190	.000	.059	.150	.116
-	Apr	.057	.000	.385	.090	.066	.028	.318	.123
SE		.052	.023	.053	.059	.023	.022	.057	.057
p		<.85	<.04	<.26	<.18	<.04	<.38	<.45	<.98

[1]If a cow had a disorder, the incidence of the disorder was 1, whereas if the cow did not have a certain disorder, the incidence of that disorder was 0.
[2]Hypocalcemia = 1 if the serum iCa concentration was 4.00 mg/dl or less.

Table 6-7d. Effects of parity (L) on incidence of hypocalcemia (HC), parturient paresis (PP), and other periparturient disorders[1].

L	HC[2]	PP	Retained placenta	Dystocia	Displaced abomasum	Ketosis	Metritis	Mastitis
1	.029	.000	.051	.324	.012	.002	.145	.110
2	.211	.002	.195	.152	.023	.033	.141	.174
3	.342	.013	.257	.060	.031	.036	.171	.153
4	.524	.105	.252	.047	.038	.062	.192	.250
5	.630	.197	.306	.113	.055	.016	.343	.130
SE	.040	.018	.040	.022	.018	.017	.045	.041
P	<.01	<.01	<.01	<.01	<.51	<.05	<.06	<.13

[1]If a cow had a disorder, the incidence of the disorder was 1, whereas if the cow did not have a certain disorder, the incidence of that disorder was 0.
[2]Hypocalcemia = 1 if the serum iCa concentration was 4.00 mg/dl or less.

Table 6-7e. Effects of interaction between parity (L) and prepartal dietary cation-anion difference (DCAD) on incidence of hypocalcemia (HC), parturient paresis (PP), and other peripartal disorders[1].

DCAD	L	HC[2]	PP	Retained placenta	Dystocia	Displaced abomasum	Ketosis	Metritis	Mastitis
+	1	.059	.000	.083	.303	.009	.000	.174	.127
+	2	.375	.000	.218	.207	.047	.001	.115	.214
+	3	.594	.027	.206	.120	.062	.058	.170	.100
+	4	.618	.143	.315	.076	.075	.089	.188	.236
+	5	.858	.280	.267	.103	.000	.001	.393	.167
-	1	.000	.000	.018	.346	.016	.006	.117	.093
-	2	.047	.003	.172	.096	.000	.066	.167	.134
-	3	.090	.000	.307	.001	.000	.015	.172	.207
-	4	.430	.068	.190	.017	.000	.035	.197	.264
-	5	.402	.114	.344	.123	.110	.031	.292	.093
SE		.058	.026	.054	.066	.025	.024	.062	.062
P		<.01	<.01	<.28	<.38	<.02	<.06	<.73	<.53

[1]If a cow had a disorder, the incidence of the disorder was 1, whereas if the cow did not have a certain disorder, the incidence of that disorder was 0.
[2]Hypocalcemia = 1 if the serum iCa concentration was 4.00 mg/dl or less.

Table 6-8a. Effects of prepartal dietary cation-anion difference (DCAD) on milk production and milk composition.

DCAD	Milk yield (kg/d)	FCM[1] (kg/d)	Protein (%)	Protein yield (kg/d)	Fat (%)	Fat yield (kg/d)	SCC[2]
+	37.6	36.1	3.03	1.13	3.74	1.41	3.19
-	37.4	36.1	3.04	1.12	3.77	1.41	3.03
SE	.4	.5	.02	.01	.05	.03	.11
P	>.25	>.25	>.25	>.25	>.25	>.25	>.25

[1]4% Fat-corrected milk (kg) = .4 X (kg of milk) + 15 X (kg of milk fat) (NRC, 1989).

[2]Somatic cell count score ranges from 0 to 9 (DRPC, 1987).

6-8b. Effects of the month of calving (M) on milk production and milk composition.

M	Milk yield (kg/d)	FCM[1] (kg/d)	Protein (%)	Protein yield (kg/d)	Fat (%)	Fat yield (kg/d)	SCC[2]
Jan	37.9	36.0	2.99	1.13	3.65	1.40	2.76
Feb	37.8	36.5	3.00	1.13	3.71	1.42	3.18
Mar	35.9	34.7	3.07	1.10	3.82	1.37	2.87
Apr	38.4	37.4	3.09	1.16	3.84	1.45	3.63
SE	.7	1.0	.03	.02	.10	.05	.12
P	>.10	<.15	<.25	>.25	>.25	>.25	>.25

[1]4% Fat-corrected milk (kg) = .4 X (kg of milk) + 15 X (kg of milk fat) (NRC, 1989).

[2]Somatic cell count score ranges from 0 to 9 (DRPC, 1987).

whereas for cows that calved in February and April, protein yield and milk yield were higher for the +DCAD group (Table 6-8c). Concentrations of protein decreased as parity increased. Concentrations of fat in milk increased from first parity through the third parity, but tended to decrease in cows of the fourth and fifth parity (Table 6-8d). Protein yield, fat yield, milk yield, fat-corrected milk yield (FCM) and somatic cell count scores all increased as parity increased up to the fourth parity, and tended to be lower for the fifth parity. There were interactions between parity and treatment on protein yield, fat yield, milk yield and FCM yield (Table 6-8e). Milk production of cows in the second, third and fourth parity was higher (4.5%) for the -DCAD group than for the +DCAD group. However, milk yield, FCM, and yields of milk components were all lower for the fifth parity cows on the -DCAD diet, because 3 cows in this category had very low production.

Total milk and components production during the first 7 mo of the experiment and the projected 305-d production were not affected by dietary treatments (Table 6-9a). Only projected 305-d protein yield and milk yield were higher for cows that calved in February and April (Table 6-9b). Total milk yield, protein yield, fat yield and fat-corrected milk all increased as parity increased up to fourth parity (Table 6-9c). The projected milk yield, protein yield and fat yield were highest for parity two and three. Total milk yield,

Table 6-8c. Effects of interaction between the month of calving (M) and prepartal dietary cation-anion difference (DCAD) on milk production and milk composition.

DCAD	M	Milk yield (kg/d)	FCM[1] (kg/d)	Protein (%)	Protein yield (kg/d)	Fat (%)	Fat yield (kg/d)	SCC[2]
+	Jan	37.4	35.5	3.00	1.12	3.65	1.39	2.83
+	Feb	38.2	37.1	3.00	1.14	3.74	1.45	3.10
+	Mar	35.1	34.0	3.04	1.06	3.85	1.36	2.78
+	Apr	39.8	37.8	3.10	1.21	3.72	1.44	4.06
-	Jan	38.4	36.4	2.98	1.14	3.64	1.42	2.69
-	Feb	37.3	35.7	3.01	1.11	3.69	1.40	3.26
-	Mar	36.7	35.4	3.09	1.12	3.79	1.38	2.96
-	Apr	37.0	36.9	3.08	1.12	3.96	1.45	3.21
SE		.8	1.1	.03	.06	.05	.06	.24
P		>.01	>.25	>.25	<.05	>.25	>.25	>.25

[1]4% Fat-corrected milk (kg) = .4 X (kg of milk) + 15 X (kg of milk fat) (NRC, 1989).

[2]Somatic cell count score ranges from 0 to 9 (DRPC, 1987).

Table 6-8d. Effects of parity (L) on milk production and milk composition.

L	Milk yield (kg/d)	FCM[1] (kg/d)	Protein (%)	Protein yield (kg/d)	Fat (%)	Fat yield (kg/d)	SCC[2]
1	30.3	28.9	3.09	.93	3.69	1.12	3.07
2	38.3	37.3	3.05	1.16	3.82	1.46	2.60
3	39.7	39.0	3.05	1.20	3.86	1.54	2.79
4	39.9	38.6	3.00	1.19	3.77	1.50	3.63
5	39.3	36.9	2.99	1.16	3.64	1.42	3.45
SE	.5	.6	.02	.01	.07	.03	.14
P	<.01	<.01	<.01	<.01	<.01	<.01	<.01

[1]4% Fat-corrected milk (kg) = .4 X (kg of milk) + 15 X (kg of milk fat) (NRC, 1989).

[2]Somatic cell count score ranges from 0 to 9 (DRPC, 1987).

Table 6-8e. Effects of interaction between parity (L) and prepartal dietary cation-anion difference (DCAD) on milk production and milk composition.

DCAD	L	Milk yield (kg/d)	FCM[1] (kg/d)	Protein (%)	Protein yield (kg/d)	Fat (%)	Fat yield (kg/d)	SCC[2]
+	1	30.7	29.1	3.09	.94	3.67	1.13	3.21
+	2	37.3	36.2	3.05	1.12	3.79	1.41	2.65
+	3	39.1	37.4	3.05	1.19	3.75	1.47	2.96
+	4	39.2	37.7	2.99	1.16	3.69	1.46	3.58
+	5	42.0	40.2	2.99	1.24	3.82	1.59	3.55
-	1	29.8	28.5	3.09	.91	3.71	1.11	2.93
-	2	39.3	38.4	3.06	1.19	3.85	1.51	2.55
-	3	40.4	40.5	3.06	1.22	3.98	1.62	2.63
-	4	40.5	38.6	3.01	1.21	3.84	1.55	3.68
-	5	36.6	33.7	2.99	1.08	3.47	1.26	3.36
SE		.7	.9	.03	.02	.09	.04	.20
P		<.01	<.01	>.25	<.01	>.25	<.01	>.25

[1]4% Fat-corrected milk (kg) = .4 X (kg of milk) + 15 X (kg of milk fat) (NRC, 1989).

[2]Somatic cell count score ranges from 0 to 9 (DRPC, 1987).

Table 6-9a. Effects of prepartal dietary cation-anion difference (DCAD) on total milk production (kg) during the first 7 months of the experiment and predicted 305-d milk production (kg).

DCAD	Milk yield	Protein yield	Fat yield	FCM[1]	Predicted 305-d Milk	Predicted 305-d Protein	Predicted 305-d Fat	SCC[2]
+	5596.4	168.4	212.7	4766.0	8608.9	257.4	318.5	3.34
-	5542.1	167.6	212.0	4731.5	8629.0	259.2	324.6	3.18
SE	87.0	2.5	4.4	71.7	107.7	3.1	5.9	.14
P	<.63	<.80	<.90	<.71	<.88	<.64	<.43	<.37

[1]4% Fat-corrected milk (kg) = .4 X (kg of milk) + 15 X (kg of milk fat) (NRC, 1989).

[2]Somatic cell count score ranges from 0 to 9 (DRPC, 1987).

Table 6-9b. Effects of the month of calving (M) on total milk production (kg) during the first 7 months of the experiment and predicted 305-d milk production (kg).

M	Milk yield	Protein yield	Fat yield	FCM[1]	Predicted 305-d Milk	Protein	Fat	SCC[2]
Jan	5890.7	178.6	226.7	5035.2	8613.8	260.3	320.5	2.84
Feb	5599.0	168.8	213.7	4771.5	8654.6	260.2	319.9	3.42
Mar	5314.1	160.5	201.6	4533.4	8301.3	249.0	314.8	2.97
Apr	5473.2	164.4	207.2	4655.0	8906.0	263.7	331.2	3.82
SE	201.0	5.8	10.3	165.5	241.6	7.0	13.6	.32
P	<.17	<.14	<.35	<.15	<.05	<.08	<.63	<.03

[1]4% Fat-corrected milk (kg) = .4 X (kg of milk) + 15 X (kg of milk fat) (NRC, 1989).

[2]Somatic cell count score ranges from 0 to 9 (DRPC, 1987).

Table 6-9c. Effects of parity (L) on total milk production (kg) during the first 7 months of the experiment and predicted 305-d milk production (kg).

M	Milk yield	Protein yield	Fat yield	FCM[1]	Predicted 305-d Milk	Protein	Fat	SCC[2]
1	4610.0	140.4	171.2	3951.1	8436.8	255.8	308.1	3.12
2	5777.9	175.0	225.1	4936.5	8963.9	269.6	340.6	2.76
3	5836.8	177.7	228.8	5000.0	8758.8	264.1	334.9	2.92
4	5919.3	177.8	228.4	5035.5	8491.1	252.8	320.2	3.76
5	5702.4	169.3	208.2	4820.6	8444.1	249.1	304.2	3.74
SE	127.0	3.6	6.5	140.5	156.5	4.4	8.6	.20
P	<.01	<.01	<.01	<.01	<.01	<.01	<.01	<.01

[1]4% Fat-corrected milk (kg) = .4 X (kg of milk) + 15 X (kg of milk fat) (NRC, 1989).

[2]Somatic cell count score ranges from 0 to 9 (DRPC, 1987).

protein yield, fat yield and the projected production were all higher for the -DCAD group in the second, third and fourth parity (Table 6-9d), but lower for the -DCAD group in the fifth parity than for the +DCAD group.

Discussion

The effect of diets with -DCAD in relation to prevention of PP has been studied previously in Norway (Ender, 1962; Dishington, 1975; Dishington et al., 1981) and in North America (Block, 1984; Oetzel et al., 1988) with relatively small numbers of cows. The present study examined the effects of diet with -DCAD on a large commercial dairy. Ammonium chloride, $(NH_4)_2SO_4$ and $MgSO_4$ were used to make the treatment diet negative in DCAD. It was indicated that a diet with negative DCAD and a high Ca concentration was more effective in prevention of PP and hypocalcemia (Ender and Dishington, 1970; Oetzel et al., 1988). That was why the diet with negative DCAD was formulated with a high Ca content, whereas the +DCAD diet was formulated to be as close as possible to NRC (1989) recommendations with the feedstuffs available. Because Ca contents of the forages were slightly higher than estimated initially, the Ca content of the +DCAD diet turned out to be higher than formulated, but is still within the range recommended by Jorgensen (1974). There were minor differences in other minerals, but a major difference in DCAD (50 meq/kg vs. -247 meq/kg DM) between the two diets due to

Table 6-9d. Effects of interaction between parity (L) and prepartal dietary cation-anion difference (DCAD) on total milk production (kg) during the first 7 months of the experiment and predicted 305-d milk production (kg).

DCAD	L	Milk yield	Protein yield	Fat yield	FCM[1]	Predicted 305-d Milk	Protein	Fat	SCC[2]
+	1	4656.7	142.2	172.4	3996.4	8514.6	258.2	309.9	3.28
+	2	5540.8	167.2	213.6	4725.3	8679.7	260.0	325.8	2.85
+	3	5681.5	172.8	215.9	4864.4	8605.6	259.0	320.2	3.03
+	4	5916.9	177.2	229.9	5024.9	8331.1	247.7	311.7	3.68
+	5	6186.4	183.0	231.5	5219.1	8913.6	261.8	325.1	3.86
-	1	4563.2	138.7	170.0	3905.8	8359.0	253.3	306.2	2.95
-	2	6015.0	182.8	236.5	5147.9	9248.0	279.2	355.4	2.68
-	3	5992.0	182.6	241.6	5135.7	8912.0	269.2	349.7	2.81
-	4	5921.9	178.4	226.9	5046.1	8651.1	257.8	326.6	3.85
-	5	5218.4	155.6	184.8	4446.7	7974.6	236.5	283.8	3.62
SE		175.8	5.1	9.0	144.8	216.4	6.1	11.9	.28
P		<.01	<.01	<.01	<.01	<.02	<.01	<.04	<.89

[1]4% Fat-corrected milk (kg) = .4 X (kg of milk) + 15 X (kg of milk fat) (NRC, 1989).

[2]Somatic cell count score ranges from 0 to 9 (DRPC, 1987).

the chloride and sulfate salts added to the diet to form the -DCAD. The CP concentration of the two diets was similar because fermented ammoniated whey was used to provide nitrogen in the +DCAD diet to balance the nitrogen from ammonium salts in the -DCAD diet.

Even though a complete blood gas analysis was not conducted, a higher serum concentration of Cl and lower concentration of total HCO_3 were observed in cows fed the diet with -DCAD, indicating a tendency for metabolic acidosis. These results were in agreement with others (Kendall et al., 1969; Vagg and Payne, 1970). Fredeen et al. (1988a) also showed that goats fed acidogenic diets had metabolic acidosis. We also showed in another study (Chapter 4) that cows fed ammonium salts had reduced blood pH and increased urinary titratable acidity, ammonium concentration and urine acid concentrations.

Serum concentrations of iCa and total Ca were higher for the cows fed the -DCAD diet than for the cows fed the +DCAD diet. The ratio of iCa to total Ca concentrations appeared to be similar for both treatment groups. Others have reported increased concentrations of total plasma Ca in dairy cows (Block, 1984), and in goats (Fredeen et al., 1988a,b), when diets with -DCAD were fed. Oetzel et al. (1988) showed that diets supplemented with a mixture of NH_4Cl and $(NH_4)_2SO_4$ increased plasma concentrations of both iCa and total Ca, but not the ratio of the two forms of Ca around parturition.

Changes in blood pH may affect the ratio of iCa to total Ca in the blood (Moore, 1970). In another study (Chapter 4), we observed increased concentrations of blood iCa but not concentrations of plasma total Ca, with a reduced blood pH in cows fed ammonium salts. In the present study, serum pH was similar for both treatment groups (Table 6-5a). The amount of acidogenic salts fed was somewhat lower in this study than that in the other study (Chapter 4). It is possible that blood pH was not changed enough to change the ratio of iCa to total Ca in the plasma.

Incidence of PP and hypocalcemia was significantly lower in cows fed the diet with -DCAD in this study, confirming the results of previous studies (Dishington, 1975; Block, 1984; Oetzel et al., 1988). Serum concentrations of iCa, total Ca, and P were higher at parturition, indicating that cows were better able to maintain serum Ca, total Ca, and P status at parturition after being fed diets with -DCAD during the late gestational period. The exact mechanism involved is not fully understood currently. The negative DCAD probably affects Ca metabolism indirectly by inducing metabolic acidosis.

Blood Ca concentrations depend on the balance between Ca input to and Ca output from the exchangeable Ca pool (Ramberg et al., 1975). Output of Ca into milk was similar for cows with PP and cows not affected by PP (Hibbs et al., 1951). Total input of Ca into the blood Ca pool depends mainly on Ca absorbed from the intestine and Ca mobilized from bone

(Ramberg et al., 1975). Conflicting results were reported for the effect of negative DCAD on Ca absorption. Ender and Dishington (1970) showed that feeding prepartum diets supplemented with acidogenic salts ($CaCl_2$, $Al_2(SO_4)_3$ and $MgSO_4$) increased Ca absorption with a resulting positive Ca balance around parturition. Acidogenic diets also increased Ca absorption in sheep and goats (Braithwaite, 1972; Fredeen et al., 1988a,b). Lomba et al. (1978) summarized data from a number of experiments and indicated that acidogenic diets increased Ca absorption only when cows were on positive Ca balance. This implied that -DCAD increased Ca absorption only when Ca intake was high. However, Leclerc and Block (1989) did not find any difference in apparent Ca absorption among cows fed diets with DCAD of 349, 121, 105 or 62 meq/kg DM (with Ca intake of 149 g to 188 g/cow/d) during the dry period. The diets with less positive DCAD resulted in slightly reduced apparent Ca absorption immediately before parturition. It seemed that the effect of negative DCAD on Ca absorption from the digestive tract was minor. Increased Ca mobilization from bones may be of key importance for the prevention of PP with -DCAD. The plasma concentration of hydroxyproline, an indicator of bone resorption was higher in cows fed diets with negative DCAD (Block, 1984). Vagg and Payne (1970) showed that Ca mobilization rate increased in sheep fed NH_4Cl. This was further supported by Fredeen et al. (1988b), who found that goats fed diets with HCl added had

increased bone resorption rates. In another study (Chapter 4), we also showed that cows fed diets with -DCAD (with NH_4Cl and $(NH_4)_2SO_4$ added) maintained higher blood Ca concentrations than cows fed the control diet, when Na_2EDTA solution was infused intravenously.

Acidosis increased Ca mobilization from bone by releasing Ca from amorphous Ca phosphates and Ca carbonates on bone surfaces (Termine and Posner, 1966; Bushinsky et al., 1985). Acidosis also may increase cell-mediated bone resorption (Mundy and Raisz, 1981). Fredeen et al., (1988b) recently suggested that an acidogenic diet increased the turnover rate of available Ca pool. Vagg and Payne (1970) indicated that the size of Ca pool also may be increased by acidogenic diets.

Serum concentrations of iCa, total Ca and P decreased as parity increased. Old cows were less efficient in absorbing Ca and less capable of mobilizing Ca from bone (Ramberg et al., 1975). However, there also was a dietary treatment by parity interaction on serum concentrations of iCa, total Ca, and P. With each mineral, serum concentrations declined less as parity increased in the -DCAD group than in the +DCAD group (Table 6-5e). The lower HCO_3 and higher anion gap in cows with higher parity suggested that more older cows tended to have metabolic acidosis.

There were no differences between the two treatment groups in body condition scores. This suggested that the diet with -DCAD did not reduce feed intake. Horst and Jorgensen

(1973) observed severe depression of feed intake of sheep fed NH_4Cl capsules. In more recent studies with total mixed rations acidogenic salts ($CaCl_2$, $Al_2(SO_4)_3$ and $MgSO_4$; NH_4Cl and $(NH_4)_2SO_4$) used to make negative DCAD diets did not depress feed intake (Block, 1984; Oetzel et al., 1988). Edema scores were similar for both treatment groups, indicating the consumption of the anionic salts did not increase the severity of udder edema.

Incidence of retained placenta, displaced abomasum, dystocia, metritis and mastitis was similar in both groups. Many cows suffered some degree of hypocalcemia at parturition (Table 6-7a-e). Severe hypocalcemia causes PP. In this study serum iCa concentrations in PP cows were below 3 mg/dl. In an epidemiological study, Curtis et al. (1983) found strong associations between PP and dystocia, retained placenta, displaced abomasum, ketosis, metritis and mastitis. They indicated that subclinical hypocalcemia could disrupt functions of smooth muscles of the uterus and skeletal muscles, resulting in other problems such as dystocia, retained placenta, and displaced abomasum (Curtis et al., 1983). However, there might be some threshold of Ca concentration, below which risk of other disorders increases. Oetzel et al. (1988) defined hypocalcemia as iCa < 4 mg/dl. Whether other functions are affected by this degree of hypocalcemia is not clear. More research is needed to define

the relationship between blood concentrations of iCa and total Ca and incidence of other metabolic disorders.

The main effect of treatment was not significant on daily production measured on monthly test days, total milk production during the first 7 mo of the experiment, or the projected 305-d production. However, examination of the parity by treatment interaction showed cows in the second through the fourth parity produced more FCM (4.9% increase) when fed prepartal diet with -DCAD than when the diet with +DCAD was fed, indicating some beneficial effect of the -DCAD diet. The calving dates of the cows on this study spread over a period of 4 mo,; therefore, days in lactation varied from 90 d to 220 d when evaluation of milk production data was done. Cows were culled for health or reproductive purposes during the course of the experiment. Cows that were culled within the first month postpartum had no production data for statistical analysis. Cows culled at a later date also resulted in missing values. This practice might mask the beneficial effects of the diet with -DCAD, because the comparison on production was based only on the healthy cows or cows that were not culled. Block (1984) observed a 7% increased in whole lactation milk production with 20 cows. The production data of whole lactations will be evaluated when all lactations are complete.

In summary, feeding prepartal diet with -DCAD increased serum concentrations of iCa, Ca, P and decreased total

bicarbonate, and reduced the incidence of hypocalcemia and PP at parturition, but had no effect on the incidence of other periparturient disorders. Milk production of cows of second through the fourth parity was higher when fed a prepartum diet with -DCAD than fed a diet with +DCAD. Results of this experiment strongly indicate that feeding a diet with -DCAD during the 3 to 4 wk before parturition will have a major beneficial effect on health and possibly the productivity of dairy cattle.

CHAPTER 7
GENERAL DISCUSSION AND CONCLUSIONS

Parturient paresis is one of the most serious metabolic diseases in dairy cows. Even though treatment with intravenous injection of Ca solutions is relatively effective, veterinary costs and labor involved in taking care of sick cows causes losses for dairy producers. This disease also reduces the production potential and increases the risk of other diseases, resulting in additional cost and losses indirectly (Curtis et al., 1984). Therefore, the best approach is to prevent the occurrence of PP.

Significant progress has been made in the last 3 decades in the understanding of Ca metabolism of dairy cows, but the exact cause and mechanism of PP is still uncertain. It is agreed generally that reduced Ca input into the exchangeable Ca pool at parturition is responsible for severe hypocalcemia. Some studies emphasized the interruption of Ca absorption from the intestine, whereas others put the role of depressed Ca mobilization from bone before Ca absorption as the primary cause of hypocalcemia at parturition. It appears that both may be depressed in cows with PP. High Ca intake prepartum has been blamed for the temporary unresponsiveness of intestine and bone to PTH and $1,25(OH)_2D_3$ at parturition. However, in the original studies by Boda and Cole (1954), and

Boda (1956), the high Ca diet contained alfalfa hay, which likely had a very positive DCAD. The preventive effect of the low Ca diet may be due in part to its less positive DCAD. Furthermore, preventive effects were observed only when Ca intake was limited to less than 20 g/cow/d. Such low rates of Ca intake are difficult to achieve with most conventional dietary regimens (Jonsson, 1978). Many forages typically are quite high in Ca content (NRC, 1989). One may have to restrict the forage intake or the total dietary intake. This may predispose the cow to digestive disorders and ketosis, and reduce the capability of the cow to reach her production potential postpartum (Verdaris and Evans, 1974). Additionally, when not enough attention is given to the amount of feed offered and the number of cows fed in a group, total ration and total Ca intakes may be greater than prescribed. More effective and practical preventive methods are needed. Therefore, the objectives of our studies were to study Ca metabolism and the dietary factors potentially influencing Ca metabolism and search for new methods and improvement on the existing measures for the prevention of PP.

Theoretically, late gestation cows (1 to 2 mo prepartum) should provide better experimental models for testing the effectiveness of any methods for PP prevention. However, the difficulty in predicting calving date, other health and management problems associated with calving make it difficult to control experimental conditions. The ability to maintain

normal blood concentrations of Ca when the demand for was increased suddenly is related inversely to the susceptibility of dairy cows to PP. Thus, in the initial studies, nonlactating and nonpregnant cows were used to test the effect of dietary manipulations on the ability of dairy cows to maintain normal blood concentrations of Ca when sudden increase in the demand for Ca at parturition was simulated by intravenous infusion of EDTA solution.

Although responses of cows to EDTA infusion was studied in three of the experiments, there were two major protocol differences between the first experiment (Chapter 3) and later experiments (Chapter 4 and 5). In the first experiment (Chapter 3), EDTA-free Ca determined according to Gindler and King (1972) included Ca bound to protein, in addition to iCa. However, it is more desirable to determine the blood concentration of iCa, the physiologically active form of Ca. A Nova 8 Calcium Analyzer was used in the later experiments for more accurate and rapid determination of blood or serum concentrations of iCa. Additionally, the endpoint of EDTA infusion in later experiments (Chapter 4 and 5) also was different from the first experiment (Chapter 3). In the first experiment (Chapter 3), infusion was terminated for all cows in a group when one of the cows showed signs of hypocalcemia, allowing the examination of the response of cows to the infusion of an equal amount of EDTA solution. In the later experiments, all cows in a group were infused simultaneously.

A blood sample was taken at the same time from all cows in the group when one of the cows showed signs of hypocalcemia. The infusion of EDTA continued for each cow until she showed signs of hypocalcemia. In this way, both the response of cows to the infusion of an equal volume of EDTA solution, and the maximal tolerance of cows to the infusion of EDTA solution also could be evaluated. Information so obtained all gave indications of cows' ability to mobilize Ca and maintain normal blood Ca concentration when the demand for Ca was increased suddenly.

In experiment 1 (Chapter 3), it was shown that increasing dietary protein intake from 11% to 19% DM did not affect Ca metabolism or the response to intravenous infusion of EDTA solution. This was different from responses reported for some nonruminant species. The results suggested that manipulation of dietary protein intake was unlikely of any value in prevention of PP.

A mixture of NH_4Cl and $(NH_4)_2SO_4$ induced mild metabolic acidosis, and increased the ability of cows to maintain normal blood Ca concentration when demand for Ca was increased by intravenous infusion of EDTA solution (Chapter 4). These results and those from Oetzel et al. (1988) demonstrated that the ammonium salts were effective in reducing DCAD and potentially will prevent PP.

Increasing Mg intake in cows fed diets with -DCAD and high Ca concentrations did not affect the response to

intravenous infusion of EDTA solution (Chapter 5). Even though Mg absorption may be depressed by high Ca intake, supplementation of Mg above requirements did not appear necessary. In the same study, $MgSO_4$ appeared to be somewhat less acidogenic than $(NH_4)_2SO_4$, because replacing $(NH_4)_2SO_4$ with $MgSO_4$ tended to increase blood pH and decrease urinary excretion of ammonium and net acid concentration.

The field study with 510 cows on a commercial dairy demonstrated that diets with -DCAD reduced the incidence of PP. Serum concentrations of iCa and total Ca were higher at parturition in cows fed the diet with -DCAD and much higher than recommended Ca content. These results contradict the theory that high Ca intake prepartum was the primary cause of PP, because incidence of PP was lower for the cows fed the diet with -DCAD and twice the Ca concentration of diet with +DCAD. Others also suggested that high Ca intake may be beneficial for the prevention of PP in cows fed diets with -DCAD (Ender and Dishington, 1970; Oetzel et al., 1988). Subclinical acidosis was induced by feeding diets with -DCAD in our studies (Chapter 4,5), in agreement with the results of others (Vagg and Payne, 1970; Fredeen et al., 1988a). These results indirectly support a hypothesis that the fundamental cause of PP may be parturient alkalosis, which may be caused by hyperventilation at parturition (Craige and Stoll, 1947; Reynolds, 1970). Serum concentrations of iCa, total Ca, and P decreased, but incidence of hypocalcemia and PP increased as

parity increased. However, serum concentrations of iCa, total Ca, and P declined less, incidence of hypocalcemia and PP increased less with increasing parity for cows fed the -DCAD diet than for cows fed the +DCAD diet. These further prove the effectiveness of the diet with -DCAD.

In conclusion, the dietary protein intake did not affect Ca metabolism in dairy cows. Supplementation of acidogenic salts increased the cows' ability to maintain normal blood Ca concentrations when the demand for Ca was increased suddenly by EDTA infusion. Increasing Mg intake above requirement for cows fed acidogenic diets did not affect the response of blood iCa concentrations to Na_2EDTA infusion. The field study confirmed that diets with -DCAD could be used on a practical basis to raise serum concentrations of iCa and total Ca at parturition, and reduce incidence of PP and hypocalcemia.

Results from the experiments presented in this dissertation and results of others (Ender et al., 1962; Dishington, 1975; Block, 1984; Oetzel et al., 1988) established that diets with -DCAD induced metabolic acidosis, increased cows ability to maintain normal blood Ca concentrations and reduced the incidence of PP. Further research is needed to clarify the role of acid-base balance on Ca mobilization from bone (including chemical exchange and cell mediated bone resorption) and activities of Ca-regulating hormones in cows. Results from other species may not apply because PP only exists in cows. The study in which dietary

protein was varied (Chapter 3) certainly shows that results from one species (eg., man and rats) can not be transferred necessarily to another (eg., the dairy cow) without careful study. The hypothesis of alkalosis as a primary cause of PP needs to be evaluated. On the practical aspect, further studies are needed to establish the optimal range of DCAD, that is effective in preventing PP. Safety and effectiveness of different anionic salts that can be used to build diets with negative DCAD need to be tested. The optimal length of time for feeding a diet with -DCAD also needs further evaluation.

Further study also is needed to examine the relationship between blood Ca concentrations at parturition and other periparturient disorders.

APPENDIX

Appendix 3-1. Least squares analysis of variance for Chapter 3 (I). Square (S), cow (C), period (P), treatment (T) and their interactions were in the model with sampling time (h) as an independent continuous variable[1].

		Pooled regression on h					Partial regression on h		
Source	df	EDTA-free Ca	Total Ca	EDTA-Bound Ca	Source	df	EDTA-free Ca	Total Ca	EDTA-Bound Ca
S	1	65.72***	24.59**	9.83**	S	1	165.72***	24.68**	9.85
C(S)	4	9.62	23.47	18.57	C(S)	4	9.62	23.44	18.56
P	2	22.58**	138.58***	112.99***	P	2	22.58**	138.60***	112.81***
T	2	16.91	11.88	7.24	T	2	1.11	.55	.18
S*P	2	4.07	9.80	3.45	S*P	2	4.07	9.76	3.45
S*T	2	11.94	2.82	4.33	S*T	2	11.94	2.85	4.34
Res1[2]	3	13.55	5.25	7.09	Res1[2]	3	13.55	5.27	7.06
h	1	177.00***	9.66***	103.32***	h*T	2	177.86***	9.88**	104.20***
h*h	1	113.51***	.15*	104.77***	h*h*t	2	114.86***	.31	106.08***
h*h*h	1	72.16**	.86**	88.30**	h*h*h*t	2	73.53**	.91	89.72**
h*h*h*h	1	49.62**	2.47***	73.84**	h*h*h*h*t	2	50.86**	2.47**	75.26**
h*h*h*h*h	1	36.55**	4.65***	62.99**	h*h*h*h*h*t	2	35.63**	3.66**	64.40**
Res2[3]	488	231.80	319.41	499.36	Res2[3]	478	228.00	314.55	491.00
dfe[4]			486	486				476	476

[1]Type III sum of squares are listed in the table.
[2]Residual 1 is used to test the effect of P, T, P*S T*S.
[3]Residual 2 is used to test the effect of D, P*D T*D P*S*D T*S*D.
[4]degrees of freedom for Res2 when different from the general df for Res2.
*P < .10; **P < .05; ***P < .01.

Appendix 3-2. Heterogeneity of regression of EDTA-free Ca, total Ca and EDTA-bound Ca on sampling time[1].

	EDTA-free Ca		Total Ca		EDTA-Bound Ca	
	df	Residual	df	Residual	df	Residual
Pooled regression	488	231.80	486	319.41	486	499.36
Partial regression	478	228.00	476	314.55	476	491.00
Difference[2]	10	3.80	10	4.86	10	8.36

[1]Type III sum of squares are listed in the table.

[2]The difference mean squares were tested by the error mean squares from the partial regression.

Appendix 4-1. Least squares analysis of variance for Chapter 4. Block (B), cow (C), period (P), treatment (T) and day of sampling (D), and their interactions were in the mathematical model[1].

Source	df	Plasma P	P/Cr[2]	FE[3] P	Plasma Ca	Ca/Cr[2]	FE[3] Ca	Plasma Mg	Mg/Cr[2]	FE[3] Mg	TA[4]	NAC[5]	NH_4
B	1	.219	13.12	.02	.001	.0	6.8	.009	.18	8.37	173.3	73.0	478.8
C(B)	6	.976	57.18	.02	.40	1.9	119.8	.073	20.90	760.92	13574.6	30016.4	9731.3
P	1	.092**	.00	.83	.00	.8	6.9***	.001	4.85	223.60	6093.0**	1915.2	1172.2
T	1	.003	.85	.50	.01	100.0***	875.4	.020	9.30	893.43*	48387.0***	174725.3***	39193.5***
P*B	1	.023	.06	.96	.00	4.5	30.1	.004***	28.08**	1667.85**	2363.2*	7124.8	1260.8
T*B	1	.251***	.72	.62	.00	1.1	1.1	.001	5.00	194.32	358.6	3046.4	1302.1
Res1[6]	4	.050	1.59	.93	.05	1.1	44.5	.033	12.00	672.76	1701.1	9198.2	6635.0**
D	2	.015	1.06	.77	.01	1.5	24.8	.004***	13.67	1045.08*	1880.5	4248.2	604.8
B*D	2	.017	.57	.85	.00	.2	3.8	.000	2.01	108.47	2578.6	5423.5	702.0
C(B)*D	12	.208	5.82	.99	.05	1.1	93.4	.032	16.52	1056.08	14752.1	25178.8	2674.2
P*D	2	.041	4.55	.33	.01	.7	9.1	.007***	2.61	105.32	1775.9	2618.4	372.3
T*D	2	.204***	2.47	.51	.02	1.8	31.0	.014***	2.50	299.53	2189.3	2956.8	1343.8
P*B*D	2	.003	5.54	.26	.00	1.8	22.0	.006**	18.65*	1050.20*	2398.6	1639.1	493.6
T*B*D	2	.019	2.84	.50	.00	.0	3.2	.001	.66	31.46	4164.6	4427.9	498.8
Res2[7]	8	.108	14.76	114.90	.04	6.3	52.9	.004	20.22	1157.82	9698.7	13272.3	2286.6

[1]Type III sum of squares are listed in the table.
[2]P/creatinine ratio, Ca/creatinine ratio, and Mg/creatinine ratio.
[3]Fractional excretion for P, Ca, and Mg repectively.
[4]Titratable acidity.
[5]Net acid concentration.
[6]Residual 1 is used to test the effect of P, T, P*B T*B.
[7]Residual 2 is used to test the effect of D, P*D T*D P*B*D T*B*D.
*P < .10; **P < .05; ***P < .01.

Appendix 4-2. Least squares analysis of variance for Chapter 4. Block (B), cow (C), period (P), treatment (T) and day of sampling (D), an their interactions were in the mathematical model[1].

Source	df	Before EQTA infusion			EQTA infusion		
		Blood iCa	Blood pH	Urine pH	Volume	Time	Rate
B	1	.025	.002	.06	28730.2	1870.6	.040
C(B)	6	.734	.022	3.09	264854.5	25992.9	.096
P	2	.328*	.014**	2.60**	8190.2	1278.1	.008
T	1	.629**	.078***	45.69***	38809.0	4128.1	.001
P*B	2	.001	.000	.03	5852.2	1040.1	.013
T*B	1	.093	.009*	.00	529.0	60.1	.000
Res1[2]	10	.197***	.006	.88	45374.5	4753.2	.015
D	2	.064***	.003***	1.07**			
B*D	2	.004	.000	.05			
C(B)*D	12	.147	.011***	2.01			
P*D	4	.005	.001*	.05			
T*D	2	.006	.000	1.27**			
P*B*D	4	.011***	.000	.04			
T*B*D	2	.037	.000	.01			
Res2[3]	8	.014	.001	.86			

[1]Type III sum of squares are listed in the table.
[2]Residual 1 is used to test the effect of P, T, P*B T*B.
[3]Residual 2 is used to test the effect of D, P*D T*D P*B*D T*B*D.
*P < .10; **P < .05; ***P < .01.

Appendix 4-3. Least squares analysis of variance of blood ionized calcium (iCa), plasma total calcium, magnesium and blood pH for Chapter 4. Block (B), cow (C), period (P), treatment (T), and their interactions were in the model with sampling time (h) as a continuous independent variable[1].

SOURCE	df	Pooled regression on h			
		iCa	tca	Mg	pH
B	1	2.81**	4.59	1.906**	.060**
C(B)	6	4.97	130.30**	2.921	.060
P	1	.49	50.71***	.586	.026
T	1	16.14**	12.97**	.413	.477**
P*B	1	1.59	.22	.345	.000
T*B	1	.63	1.79	.237	.091
Res1[2]	4	3.55***	12.92***	3.868***	.038
h	1	57.98***	1.29***	.401***	.029***
h*h	1	30.37***	1.29***	.521***	.024***
h*h*h	1	14.42***	4.36***	.472***	.016***
h*h*h*h	1	7.29***	5.95***	.378***	
h*h*h*h*h	1	3.96***	6.43***		
Res2[3]	459	28.48	86.07	10.044	.193
dfe[4]			453	454	461

SOURCE	df	Partial regression on h			
		iCa	Total Ca	Mg	pH
B	1	2.81**	4.56	1.892**	.060**
C(B)	6	4.97	130.22***	2.919	.060
P	1	.49	50.62***	.594	.026
T	1	.82	.11	.122	.095
P*B	1	1.59	.21	.339	.000
T*B	1	.63	1.85	.237	.091
Res1[2]	4	3.55	12.78***	3.883***	.038
h*T	2	58.00	1.30**	.445***	.032
h*h*T	2	30.37	1.31**	.566***	.024
h*h*h*T	2	14.44	4.37***	.497***	.016
h*h*h*h*T	2	7.36	5.96***	.391***	
h*h*h*h*h*T	2	4.07	6.43***		
Res2[3]	454	27.31	77.97	8.705	.163
			448	450	458

[1]Type III sum of squares are listed in the table.
[2]Residual 1 is used to test the effect of P, T, P*B T*B.
[3]Residual 2 is used to test the effect of D, P*D T*D P*B*D T*B*D.
[4]degrees of freedom for Res2 when different from the general df for Res2.
*P < .10; **P < .05; ***P < .01.

Appendix 4-4. Heterogeneity of regression of blood ionized calcium (iCa), plasma total Ca, magnesium and blood pH on sampling times[1].

	iCa		Total Ca		Mg		pH	
	df	residual	df	residual	df	residual	df	residual
Pooled regression	459	28.48	453	86.07	454	10.044	461	.193
Partial regression	454	27.31	448	77.97	450	8.705	458	.163
Difference[2]	5	1.17**	5	8.10**	4	1.339**	3	.030***

[1]Type III sum of squares are listed in the table.

[2]The difference mean squares were tested by the error mean squares from the partial regression.

*P < .10; **P < .05; ***P < .01.

Appendix 5-1. Least squares analysis of variance for Chapter 5. Block (B), cow (C), period (P), treatment (T), day of sampling (D), and their interactions were in the mathematical model[1].

Source	df	Plasma P	P/Cr[2]	FE[3] P	Plasma Ca	Ca/Cr[2]	FE[3] Ca	Plasma Mg	Mg/Cr[2]	FE[3] Mg	TA[4]	NAC[5]	NH_4
B	1	.02	.70*	15.4*	.43***	34.79*	396.0	.154**	5.41	171.2	300.0**	40.3	123.0
C(B)	6	1.21	.75	22.7	.20	47.32	770.0	.123	29.41	1784.9	195.6	1074B.5	11B37.6
P	2	.59	.06	2.9	.11***	122.51***	1331.4***	.007	45.05**	4284.6**	58.1	6844.5*	5615.4*
T	1	1.00**	.00	1.0	.02*	22.56*	339.B*	.016	11.B5	900.7	5.3	14525.6***	14141.9***
P*B	2	.09	.09	5.7	.00	22.22	291.4	.068	4.01	126.1	58.0	717.0	355.9
T*B	1	.2B	.02	2.1	.01	18.4B	2B9.2*	.00B	4.B9	B53.4	2.6	204.5	267.1
Resi[6]	10	2.04	.B1*	32.6	.0B**	59.53*	7B5.B*	.155***	57.22***	3564.3**	109.4	9840.4	8619.7
D	2	.0B	.10	4.3	.03**	6.50	94.5	.001	2.12	302.5	46.9*	2272.2	2229.7
B*D	2	.21	.10	3.9	.00	1.13	22.7	.002	.16	4.1	2.3	BB6.2	B00.9
C(B)*D	12	.24	.17	10.4	.06	43.10	560.5	.00B	24.41	2281.5	144.6	10664.6	9650.5
P*D	4	.20	.20	9.0	.01	19.65	256.5	.017***	12.55*	1172.0*	41.9	2622.7	2189.7
T*D	2	.0B	.00	.2	.00	24.29**	2B5.3**	.002	10.74**	756.5*	2.B	3B9.5	431.2
P*B*D	4	.17	.23	9.5	.02	5.60	77.4	.001	2.B8	134.0	57.5	9B5.6	555.2
T*B*D	2	.07	.00	.3	.00	4.00	72.6	.001	1.76	307.6	23.6	30.4	40.1
Res2[7]	20	.B6	.76	32.5	.06	50.77	667.4	.019	22.69	2191.6	153.7	10B1B.3	9652.1

[1]Type III sum of squares are listed in the table.
[2]P/creatinine ratio, Ca/creatinine ratio, and Mg/creatinine ratio.
[3]Fractional excretion for P, Ca, and Mg repectively.
[4]Titratable acidity.
[5]Net acid concentration.
[6]Residual 1 is used to test the effect of P, T, P*B T*B.
[7]Residual 2 is used to test the effect of D, P*D T*D P*B*D T*B*D.
*P < .10; **P < .05; ***P < .01.

Appendix 5-2. Least squares analysis of variance for Chapter 4. Block (B), cow (C), period (P), treatment (T) and day of sampling (D), an their interactions were in the mathematical model[1].

Source	df	Before EOTA infusion			EDTA infusion		
		Blood iCa	Blood pH	Urine pH	Volume	Time	Rate
B	1	1.06***	.0014	.713	53110.0	22387.0***	3.61***
C(B)	6	.43	.0054	1.706	322516.2***	8964.9***	.07
P	2	2.55***	.0209**	.373	444736.3***	80668.0***	12.03***
T	1	.00	.0051	.106	5504.1	108.0	.00
P*B	2	.00	.0034	.207	215752.2***	36596.3***	2.71***
T*B	1	.02	.0004	.039	4961.3	90.8	.01
Resi[2]	10	.28	.0187	.745**	47393.2		
D	2	.03	.0010	.124			
B*D	2	.03	.0000	.039			
C*D(B)	12	.14	.0069	.593			
P*D	4	.21**	.0015	.487***			
T*D	2	.05	.0015	.086			
P*B*D	4	.12	.0018	.171			
T*B*D	2	.00	.0006	.059			
Res[3]	20	.31	.011	.380			
dfe[4]			13				

[1]Type III sum of squares are listed in the table.
[2]Residual 1 is used to test the effect of P, T, P*B T*B.
[3]Residual 2 is used to test the effect of D, P*D T*D P*B*D T*B*D.
[4]degrees of freedom for error when different from the general df for error.
*P < .10; **P < .05; ***P < .01.

Appendix 5-3. Least squares analysis of variance of blood ionized calcium (iCa), plasma total calcium, magnesium and blood pH for Chapter 5. Block (B), cow (C), period (P), treatment (T), and their interactions were in the model with sampling time (h) as a continuous independent variable[1].

	Pooled regression on h					Partial regression on h				
SOURCE	df	iCa	Total Ca	Mg	pH	SOURCE	df	iCa	Total Ca	Mg	pH
B	1	19.78***	95.166***	1.795	.07	B	1	19.75***	95.25	1.780	.07
C(B)	6	7.20*	98.098**	.293	.06	C(B)	6	7.21	97.85	.294	.06
P	2	19.88***	15.211	1.360	.21	P	2	19.89***	15.17	1.367	.21
T	1	.92	1.966	.650	.10	T	1	.52	1.04	.027	.01
P*B	2	2.21	1.267	1.529	.00	P*B	2	2.22	1.26	1.544	.00
T*B	1	.14	.019	.694	.00	T*B	1	.14	.01	.700	.00
Res1[2]	10	4.34	18.880***	10.449***	.10	Res1[2]	10	4.34	18.89***	10.486***	.10
h	1	59.43***	.010	.135***	.03***	h*T	2	59.60***	1.04**	.138***	.03**
h*h	1	34.68***	2.520***	.150***	.03***	h*h*T	2	34.73***	3.48***	.163***	.03**
h*h*h	1	19.11***	4.940***	.143***	.02***	h*h*h*T	2	19.12***	5.77***	.166***	.02**
h*h*h*h	1	11.59***	5.870***	.115***		h*h*h*h*T	2	11.60***	6.61***	.142***	
h*h*h*h*h	1	7.76***	6.010***			h*h*h*h*h*T	2	7.77***	6.66***		
Res2[3]	686	133.11	106.80	5.373	.23	Res2[3]	681	132.10	105.61	5.220	.22
dfe[4]			687	685					683		

[1]Type III sum of squares are listed in the table.
[2]Residual 1 is used to test the effect of P, T, P*B T*B.
[3]Residual 2 is used to test the effect of D, P*D T*D P*B*D T*B*D.
[4]degrees of freedom for Res2 when different from the general df for Res2.
*P < .10; **P < .05; ***P < .01.

Appendix 5-4. Heterogeneity of regression of blood ionized calcium (iCa), plasma total Ca, magnesium and blood pH on sampling times[1].

	iCa		Total Ca		Mg		pH	
	df	residual	df	residual	df	residual	df	residual
Pooled regression	686	133.11	686	106.80	686	5.373	686	.23
Partial regression	681	132.10	681	105.61	682	5.220	683	.22
Difference[2]	5	1.01	5	1.19	4	.153	3	.01

[1]Type III sum of squares are listed in the table.

[2]The difference mean squares were tested by the error mean squares from the partial regression.

*P < .10; **P < .05; ***P < .01.

Appendix 6-1. Least squares analysis of variance for Chapter 6. Treatment (T), month of calving (M), parity (L) and their interactions were in the mathematical model with duration (DUR) as a continuous independent variable[1].

SOURCE	df	Total Protein	Albumin	Globulin	A/G[2]	Glucose	Total bilirubin	SUN[3]	Cr[4]	AP[5]	SGOT[6]	SGGT[7]
T	1	.04	.00	.01	.000	100.0	.16*	119.7*	1.12**	381.0	580.8	146.95
M	3	4.35***	1.09**	1.04*	.047	8507.4***	.53**	302.0**	.28	5029.0	4007.6	1666.92
T*M	3	1.39*	.59	.24	.046	768.6	.55**	75.8	.20	260.1	1004.1	3027.82
L	4	13.24***	1.22**	6.49***	1.252*	4098.0	.93***	245.6	3.78***	17856.4***	27737.2***	84520.51
T*L	4	.58	.96*	.45	.352	1793.5	.58**	274.2	.21	932.8	2379.1	2608.21
M*L	12	1.51	1.17	1.77	.918	12804.3**	1.63***	350.1	1.24**	16722.7	6355.9	3881.61
T*M*L	12	3.38	.89	2.11	.964	10111.2	1.10	454.9	.92	17242.7	14736.2	5241.21
DUR	1	.87**	.06	.49*	.107	3359.4**	.50***	6.8	.85***	1547.5	812.4	37.77
ERROR	453	102.20	49.50	73.96	40.10	260501.0	28.5	17799.7	24.56	1082032.6	428186.5	269132.43

[1]Type III sum of squares are listed in the table.
[2]Albumin/globulin ratio, g/g.
[3]Serum urea nitrogen.
[4]Creatinine.
[5]Alkaline phosphatase.
[6]Serum glutamic-oxalocetic transaminase.
[7]Serum gamma-glutamyltransferase.
*P < .10; **P < .05; ***P < .01.

Appendix 6-2. Least squares analysis of variance for Chapter 6. Treatment (T), month of calving (M), (L) and their interactions were in the mathematical model with duration (DUR) as a continuous independent variable[1].

SOURCE	DF	Na	K	Cl	iCa	Ca	pH	Mg	p	HCO3	Anion gap[2]
T	1	.11	.58	286.9***	210825.17***	59.1***	46.95***	.15	.07	352.6***	8.5
M	3	73.84	6.39***	145.6*	61953.01***	31.2***	15.62**	15.05***	14408.07***	210.5***	80.6**
T*M	3	268.50**	.64	192.4**	3206.53*	2.7	4.88	.74	1296.76**	13.0	13.6
L	4	108.99	2.74	244.0**	579478.27	203.8***	116.08***	1.91	1091.87	103.2***	116.8**
T*L	4	142.69	1.35	259.8**	55146.81	33.6***	20.67**	2.35	918.56	31.5	17.2
M*L	12	158.92	5.36	132.1	50174.05	17.8	24.10	6.88	2687.38	148.3*	174.5
T*M*L	12	282.75	5.08	189.9	16230.61	15.5	29.21	4.54	2012.76	143.2	109.7
DUR	1	6.65	.82	113.3**	106.34	2.1	.54	2.72*	46.03	11.9	77.9***
ERROR	454	12827.63	179.2	10029.6	917984.10	649.2	795.07	392.71	86094.90	3563.0	4758.3
dfe[3]					469				469		

[1]Type III sum of squares are listed in the table.
[2]Anion gap = the serum concentration of (Na meq/L + K meq/L) - (Cl meq/L + CO_3 meq/L).
[3]degrees of freedom for error when different from the general df for error.
*P < .10; **P < .05; ***P < .01.

Appendix 6-3. Least squares analysis of variance for Chapter 6. Treatment (T), Month of calving (M) and Parity (L) were the main factors in the mathematical model with duration (DUR) as a continuous independent variable[1].

SOURCE	df	Hypocalcemia (iCa < 4 mg/dl)	PP[2]	Retained placenta	Ketosis	Metritis	Mastitis	Oisplaced abomasum	Oystocia
T	1	7.495***	.221***	.010	.000	.028	.008	.015	.160
M	3	3.028***	.248***	1.526***	.060	1.579***	.505	.019	.351
M*T	3	.089	.188**	.455	.062	.346	.016	.187**	.694
L	4	18.984***	1.525***	4.337***	.198**	1.196*	.950	.072	6.261***
T*L	4	3.475***	.244**	.573	.180*	.268	.421	.269**	.606
M*L	12	2.316**	.764***	3.625***	.1956	1.416	1.039	.505**	.533
M*T*L	12	1.865	.218	1.421	.467**	2.775**	1.100	.421*	1.067
DUR	1	.000	.041	.084	.0474	.263	.035	.001	.562
ERROR	469	11.187	53.7956		9.5242	62.670	62.621	10.277	67.985

[1]Type III sum of squares are listed in the table.
[2]Parturient paresis.
*P < .10; **P < .05; ***P < .01.

Appendix 6-4. Least squares analysis of variance for milk production data of Chapter 6. Treatment (T), month of calving (M), parity (L), and their interactions were in the mathematical model with duration (DUR) and days in lactation (DIM) as a continuous independent variable[1].

SOURCE	df	Milk yield	Protein yield	Fat yield	FCM[2]	Predicted 305-d yield			SCC[3]
						Milk	Protein	Fat	
T	1	1103759.6	185.4	268.7	443721.5	147505.1	1248.9	13702.7	2.0
M	3	23908662.0	41439.8	21796.8	17361054.7	56871417.4**	40014.8*	37523.2	23.2**
T*M	3	42161058.8**	47429.0	38058.2**	30317072.6**	35365210.5	43430.8**	12957.5	14.3
L	4	773859435.0***	1562988.9***	663230.1***	544432945.7***	99695165.7***	93510.0***	434116.9***	48.8***
T*L	4	69050441.2***	186549.1***	64659.3***	50854309.6***	86830863.0***	82184.6***	219791.1***	2.8
M*L	12	136805466.2***	320921.0***	125391.7***	98794909.7***	206008710.1***	154477.3***	572794.4***	56.9**
T*M*L	12	150061911.5***	190922.7	115393.0***	98670327.2***	64794574.3	43250.8	177334.5	53.7**
OUR	1	16752914.3**	48041.2**	21817.1**	15584956.7**	14432759.7	15653.7*	54654.7	6.5*
OIM	1	180389979.1***	297962.9***	177452.3***	136682699.2**	1680509.2	2822.9	8717.2	1.5
ERROR	458	2196323523.8	5786170.3	1838569.6	1489335365.7	3238615535.4	2628941.4		1163.5

[1]Type III sum of squares are listed in the table.

[2]4% fat-corrected milk yield (kg) = .4 X (kg of milk + 15 X (kg of milk fat) (NRC, 1989).

[3]Somatic cell count score ranges from 0 to 9 (DRPC, 1987)

*P < .10; **P < .05; ***P < .01.

Appendix 6-5. Least squares analysis of variance for milk production data of Chapter 6 (Continued). Treatment (T), month of calving (M) and parity (L) were the main factors in the mathematical model with days in lactation (DIM) as a continuous independent variable[1].

SOURCE	DF	Milk yield	FCM[2] yield	Protein yield	Fat yield	Protein %	Fat %	SCC[3]
T	1	149.3	.1	.02	.24	.1	.0	8.1
M	3	5082.1*	10841.7	.67	22.04	2.7	27.1	80.9
T*M	3	3954.8***	2091.4	.39**	3.51	4.3	2.2	44.0
L	4	192148.1***	207161.3***	2.37***	12.41***	147.7***	349.1***	196.7***
T*L	4	8771.7***	14380.3***	.03***	9.24***	8.4	34.6	7.1
M*L	12	14116.7	27068.4	1.28	20.79	8.9	56.8	230.5
T*M*L	12	5879.2	8502.9	1.70	14.83	4.2	17.4	188.5
COW(T*M*L)	460	289204.6	410579.7	84.20	727.90	442.9	968.9	5194.4
DIM	1	1397.1***	106.4***	.53	3.52**	.5**	.2	.1
DIM*DIM	1	14253.4***	15142.3***	4.01**	1.47**	5.5***	24.8***	6.5**
ERROR	2018	542404.5	947505.5	200.5	2426.2	430.0	2542.8	9719.1
dfe[4]				2006	2006	2006	2006	

[1]Type III sum of squares are listed in the table.

[2]4% fat corrected milk yield (kg) = .4 X (kg of milk) + 15 X (kg of fat) (NRC, 1990).

[3]Somatic cell count score ranges from 0 to 9 (DRPC, 1987).

[4]degrees of freedom for error when different from the general df for error.

*P < .10; **P < .05; ***P < .01.

REFERENCES

Alcock, N. and I. MacIntyre. 1962. Interrelation of calcium and magnesium absorption. Clin. Sci. 22:185.

Alcock, N and I. MacIntyre. 1964. Some effects of magnesium repletion on calcium metabolism in the rat. Clin. Sci. 26:219.

Allen, L. H., Bartlett, R. S., and G. D. Block. 1979. Reduction of renal Ca absorption in man by consumption of dietary protein. J. Nutr. 109:1345.

Allen, L. H., Geoffrey, D., Block, M. S., Richard, J., Wood, M. S. and F. B. Graeme. 1981. The role of insulin and parathyroid hormone in the protein induced calciuria of man. Nutr. Res. 1:3.

Aronson, A. L. and F. A. Ahrens. 1971. The mechanism of renal transport and excretion of ethylene diamine tetraacetate with interspecies comparisons. Toxic. Appl. Pharmac. 18:1.

Association of Official Analytical Chemists. 1970. Official methods of analysis. 11th ed. Assoc. Offic. Anal. Chem., Washington DC.

Barr, A. J., J. H. Goodnight, J. P. Sall and J. T. Helwig. 1982. A user's guide to SAS 82. SAS Inst. Inc., Raleigh, NC.

Barzel, U. S. 1969. The effect of excessive acid feeding on bone. Calcified Tissue Res. 4:94.

Barzel, U. S. 1981. Parathyroid hormone, acid-base balance and calcium metabolism: interrelations and interactions. Page 251 in Disorders of mineral metabolism. Vol. III. F. Bronner and J. W. Coburn. ed. Academic Press, New York, NY.

Barzel, V. S. and J. Jowsey. 1969. The effects of chronic acid and alkali administration on bone turnover in adult rats. J. Clin. Sci. 36:517.

Beck, N. and S. Webster. 1976. Effects of acute metabolic acidosis on renal action and calcium metabolism. Am. J. Physiol. 118:1483.

Bikle, D. D., Munson, S. and J. G. Chafouleas. 1984. The role of calmodulin in 1,25-dihydroxyvitamin D regulation of calcium transport across the intestinal brush border membrane. Page 193 in Epithelial calcium and phosphate transport. F. Bronner and M. Peterlik. ed. Alan R. Liss Inc, New york, NY.

Bikle, D. D., Zolock, D. T., Morrisey, R. L. and R. H. Herman. 1978. Independence of 1,25-dihydroxyvitamin D_3-mediated calcium transport from de novo RNA and protein synthesis. J. Biol. Chem. 253:484.

Black, H. E. and C. C. Capen. 1973. Plasma calcitonin-like activity and urinary cyclic adenomonophosphate during pregnancy, parturition and lactation in cows with parturient hypocalcemia. Horm. Metab. Res. 5:297.

Block, E. 1984. Manipulating dietary anions and cations for prepartum dairy cows to reduce incidence of milk fever. J. Dairy Sci. 67:2939.

Boda, J. M. 1956. Further studies on the influence of dietary calcium and phosphorus on the incidence of milk fever. J. Dairy Sci. 39:66.

Boda, J. M. and H. H. Cole. 1954. The influence of dietary calcium and phosphorus on the incidence of milk fever in dairy cattle. J. Dairy Sci. 37:360.

Boda, J. M. and H. H. Cole. 1956. Calcium metabolism with special reference to parturient paresis (milk fever) in dairy cattle: a review. J. Dairy Sci. 39:1027

Bohatirchuk, F. 1966. Calciolysis as the initial stage of bone resorption. A stain historadiographic study. Am. J. Med. 41:836.

Braithwaite, G. D. 1972. The effect of ammonium chloride on calcium metabolism in sheep. Br. J. Nutr. 27:201.

Braun, R. K., G. A. Donovan, T. Q. Tran, J. K. Shearer, E. L. Bliss, D. W. Webb, D. K. Beede and B. Harris. 1987. Body condition scoring dairy cows as a herd management tool. Page F62 in Compendium on continuing education for the practicing veterinarian. W. M. Guterbock. ed. Veterinary Learning Systems Co. Inc., Ontario, CA.

Bronner, F., D. Pansu, A. Bosshard and J. H. Lipton. 1983. Calcium uptake by isolated rat intestinal cells. J. Cell Physiol. 116:322.

Buckley, M and F. Bronner. 1980. Calcium binding protein biosynthesis in the rat: regulation by calcium and 1,25-dihydroxyvitamin D_3. Arch. Biochem. Biophys. 202:235.

Burnell, J. M. 1971. Changes in bone sodium and carbonate in metabolic acidosis and alkalosis in the dog. J. Clin. Invest. 50:327.

Bushinsky, D. A. and R. J. Lechleider. 1987. Mechanism of proton-induced bone calcium release: Calcium carbonate dissolution. Am. J. Physiol. 253:F998.

Bushinsky, D. A., J. S. Riera, M. J. Favus and F. L. Coe. 1985. Response of serum 1,25-$(OH)_2D_3$ to variation of ionized calcium during chronic acidosis. Am. J. Physiol. 249:F361.

Capen, C. C., C. R. Cole and J. M. Hibbs. 1968. Influence of vitamin D on calcium metabolism and the parathyroid glands of cattle. Fed. Proc. 27:142.

Capen, C. C. and D. M. Young. 1967. The thyrocalcitonin: evidence for release in a spontaneous hypocalcemic disorder. Science. 157:205.

Car, C. W. 1955. Competitive binding of calcium and magnesium with serum albumin. Proc. Soc. Exp. Biol. Med. 89:546.

Care, A.D., J.-P. Bartlet and H. M. Abdel-Hafeez. 1980. Calcium and phosphorus homeostasis in ruminants and its relationship to the aetiology and prevention of parturient paresis. Page 429 in Digestive physiology and metabolism in ruminants. Y. Ruckebusch and P. Thivend. ed. AVI publishing company, Inc., Westport, Connecticut.

Care, A. D., C. W. Cooper and H. Orimo. 1968. Page 417 in Parathyroid hormone and thyrocalcitonin (calcitonin). R. V. Talmage and L. F. Belanger. ed. Excerpta medica foundation. Armsterdam. Sweden.

Care, A. D. and A. T. Van't Klooster. 1965. In vivo transport of magnesium and other cations across the wall of the gastrointestinal tract of sheep. J. Physiol. 177:174.

Chan, J. C. M. 1972. The rapid determination of urinary titratable acid and ammonium and evaluation of freeing as a method of preservation. Clin. Biochem. 5:94.

Chan, J. C. M. 1981. Nutrition and acid-base balance. Fed. Proc. 40:2423.

Contreras, P. A., R. Manston and B. F. Sansom. 1982. Calcium mobilization in hypomagnesemic cattle. Res. Vet. Sci. 33:10.

Craige, A. H. 1947. A clue to the cause of milk fever in the metabolism of the springing cow. Am. J. Vet. Res. 8:247.

Craige, A. H. and I. V. Stoll. 1947. Milk fever (Parturient paresis) as a manifestation of alkalosis. Am. J. Vet. Res. 8:168.

Curtis, R. A., J. F. Cote and J. A. Mills. 1970. An epizootiological study of parturient paresis (milk fever). Page 81 in Parturient hypocalcemia. J. J. B. Anderson. ed. Academic Press, New York, NY.

Curtis, C. R., Erb, H. N., Sniffen, C. J. and R. D. Smith. 1984. Epidemiology of parturient paresis: predisposing factors with emphasis on dry cow feeding and management. J. Dairy Sci. 67:817.

Curtis, C. R., H. N. Erb, C. J. Sniffen, R. D. Smith, P. A. Powers, M. C. Smith, M. E. White, R. B. Hillman and E. J. Pearson. 1983. Association of parturient hypocalcemia with eight periparturient disorders in Holstein cows. J. Am. Vet. Med. Assoc. 183:559.

Dairy Record Processing Center. 1987. Dairyman's DHI manual. DRPC @ Raleigh. Raleigh, NC.

Daniel, C. W. 1980. Induced hypocalcemia in cows and sheep. III. Changes in plasma magnesium levels. Br. Vet. J. 136:51.

DeLuca, H. F. 1981. Recent advances in the metabolism of vitamin D. Ann. Rev. Physiol. 43:199.

Dishington, I. W. 1975. Prevention of milk fever. Acta Vet. Scand. 16: 503.

Dishington, I. W. & J. Bjornstad. 1982. Prevention of milk fever by dietary means. Acta Vet. Scand. 23:336.

Dowdle, E. B., Schachter, D. and H. Schenker. 1960. Requirement for the active transport of calcium by the intestine. Am. J. Physiol. 198:269.

Dryerre, H and J. R. Greig. 1925. Milk fever: its possible association with derangements in the internal secretions. Vet. Rec. 5:225.

Ender, F. and I. W. Dishington. 1970. Etiology and prevention of parturient paresis puerperalis in dairy cows. Page 71 In Parturient hypocalcemia. J. J. B. Anderson. ed. Academic Press, New York, NY.

Ender, F., I. W. Dishington and Helgebostad. 1962. Parturient paresis and related forms of hypocalcemic disorders induced experimentally in dairy cows. Part II. Acta Vet. Scand. 3 (Suppl. 1):1.

Estep, H., W. Shaw, C. D. Watlington, C. Hobe, W. Holland and H. St. G. Tucker. 1968. Hypocalcemia due to reversible parathyroid hormone resistance. Clin. Res. 16:265.

Estep, H., W. A. Shaw, C. Watlington, R. Hobe, W. Holland and St. G. Tucker. 1969. Hypocalcemia due to hypomagnesemia and reversible parathyroid hormone unresponsiveness. J. Clin. Endocrinology. Met. 29:842.

Favus, M. J. 1985. Factors that influence absorption and secretion of calcium in the small intestine and colon. Am. J. Physiol. 248:G147.

Fiske, C. H. and Y. Subbarow. 1925. The colorimetric determination of phosphorus. J. Biol. Chem. 66:375.

Forman, H., T. T. Tujillo and L. Alames. 1954. The metabolism of C^{14}-labeled ethylene diamine tetraacetic acid in human beings. J. Lab. Clin. Med. 48:566.

Forman, H., M. Vier and M. Magee. 1953. The metabolism of C^{14}-labeled ethylene diamine tetraacetic acid in the rat. J. Biol. Chem. 203:1045.

Forslund, K., K. Lundstrom and L. E. Edquist. 1980. Equal calcitonin response in paretic and non-parentic cows after intravenous calcium infusion. Acta. Vet. Scand. 21:171.

Fredeen, A. H., E. J. DePeters and R. L. Baldwin. 1988a. Characterization of acid-base disturbances and the effects on calcium and phosphorus balances of dietary fixed ions in pregnant or lactating does. J. Anim. Sci. 66:159.

Fredeen, A. H., E. J. DePeters and R. L. Baldwin. 1988b. Effects of acid-bas disturbance s caused by differences in dietary fixed ion balance on kinetics of calcium metabolism in ruminants with high calcium demand. J. Anim. Sci. 66:174.

Freitag, J. J., K. J. Martin and M. B. Contrades. 1979. Evidence for skeletal resistance to parathyroid hormone in magnesium deficiency. J. Clin. Invest. 64:1238.

Garabedian, M., M. F. Hollick, H. F. Deluca and I. T. Boyle. 1972. Control of 25-hydroxy cholecalciferol metabolism by parathyroid glands. Proc. Natl. Acad. Sc. (USA) 69:1673.

Gast, D. R., R. L. Horst, N. A. Jorgensen and H. F. Deluca. 1979. Potential use of 1,25-dihydroxy vitamin D_3 in prevention of parturient paresis. J. Dairy Sci. 62:1009.

Gaynor, P. J., F. J. Mueller, J. K. Miller, N. Ramsey, J. P. Goff and R. L. Horst. 1989. Parturient hypocalcemia in Jersey cows fed alfalfa haylage-based diets with different cation to anion ratios. J. Dairy Sci. 72:2525.

Ghijsen, W. E. J. M., M. D. DeJong and C. H. Van Os. 1982. ATP-dependent calcium transport and its correlation with Ca-ATPase activity in basolateral plasma membrane of rat duodenum. Biochim. Biophys. Acta 689:327.

Gindler, E. M. and J. D. King. 1972. Rapid colorimetric determination of calcium in biplogical fluids with methylene blue. Am. J. Clin. Pathol. 58:376.

Goings, R. L., N. L. Jacobson, D. C. Beitz, E. T. Littledike and K. D. Wiggers. 1974. Prevention of parturient paresis by a prepartum calcium deficient diet. J. Dairy Sci. 57:1184.

Goodman, D. A., J. Lemann, Jr., E. J. Lennon and A. S. Relman. 1965. Production, excretion and net balance of fixed acid in patients with renal acidosis. J. Clin. Invest. 44:495.

Goodrich, R. D. & A. D. Tillman. 1966. Effects of sulfur and nitrogen sources and copper levels on the metabolism of certain minerals by sheep. J. Anim. Sci. 25:484.

Green, H. B., R. L. Horst, D. C. Beitz and E. T. Littledike. 1981. Vitamin D metabolites in plasma of cows fed a prepartum low calcium diet for prevention of parturient hypocalcemia. J. Dairy Sci. 64:217.

Hall, G. E. and A. D. Kenny. 1987. Role of carbonic anhydrase in bone resorption: effect of acetazolamide on basal and parathyroid hormone-induced bone metabolism. Calcif. Tissue Int. 40:212.

Halse, K. 1984. Calcium effects on renal conservation of magnesium in cows. Acta. Vet. Scand. 25:213.

Hansard, S. L., C. L. Comar and G. K. Davis. 1954. Effects of age upon calcium utilization and maintenance requirements in the bovine. J. Anim. Sci. 13:25.

Harrison, H. E. and H. C. Harrison. 1960. Transfer of Ca^{45} across intestinal wall in vitro in relation to the action of vitamin D and cortisol. Am. J. Physiol. 199:265.

Harrison, H. E. and H. C. Harrison. 1965. Vitamin D and the permeability of intestinal mucosa to calcium. Am. J. Physiol. 208:370.

Hegsted, M. and H. M. Linkswiler. 1981. Long term effects of level of protein intake on Ca metabolism in young adult women. J. Nutr. 111:244.

Henry, R. J., D. C. Cannon and J. W. Winkelman. 1974. Page 521 in Clinical Chemistry, Principles and techniques. 2nd ed. Harper and Row Inc, Hagerston, MD.

Hibbs, J. W. 1950. Milk fever (parturient paresis) in dairy cows - A review. J. Dairy Sci. 33:758.

Hibbs, J. W. and W. D. Pounden. 1955. Studies on milk fever. IV. Prevention by short time prepartum feeding of massive doses of vitamin D. J. Dairy Sci. 38:65.

Hibbs, J. W., W. D. Pounden and W. E. Krauss. 1951. Studies on milk fever in dairy cows. III. Further studies on the effect of vitamin D on some of the blood changes at parturition and the composition of colostrum in normal and milk fever cows. J. Dairy Sci. 34:855.

Horst, R. L. 1986. Regulation of calcium and phosphorus homeostasis in dairy cows. J. Dairy Sci. 69:604.

Horst, R. L., J. A. Eisman, N. A. Jorgensen and H. F. DeLuca. 1978. Adequate response of plasma 1,25-dihydroxyvitamin D to parturition in paretic (milk fever) dairy cows. Science. 196:662.

Horst, R. L. and N. A. Jorgensen. 1973. Effects of ammonium chloride on nitrogen and mineral balance in lactating and nonlactating goats. J. Dairy Sci. 57:683.

Horst, R. L. and T. A. Reinhardt. 1983. Vitamin D metabolism in ruminants and its relevance to the periparturient cow. J. Dairy Sci. 66:661.

Hove, K. 1986. Cyclic changes in plasma calcium and the calcium homeostatic endocrine system of the postparturient dairy cows. J. Dairy Sci. 69:2072.

Hove, K. and B. L. Hilde. 1984. Plasma calcium in the lactating cow: dependence on continuous intestinal absorption. Can. J. Anim. Sci. (suppl.1):227.

Hove, K., R. L. Horst, E. T. Littledike and D. C. Beitz. 1984. Infusions of parathyroid hormone in ruminants: hypercalcemia and reduced plasma 1,25-dihydroxyvitamin D concentrations. Endocrinology. 114:897.

Hunt, J. N. 1956. The influence of dietary sulfur on the urinary output of acid in man. Clin. Sci. 5:119.

Hurwitz, S., I. Cohen, A. Bar and S. Bornstein. 1973. Sodium and chloride requirements of the chick. Relationship to acid-base balance. Poult. Sci. 52:903.

Jackson, H. D., A. R. Pappenhagen, G. D. Goetsch and C.H. Noller. 1962. Effect of parathyroid hormone on calcium and other plasma constituent of dairy cattle near parturition. J. Dairy Sci. 45:89.

Johnson, N. E., E. N. Alcantara and H. Linkswiler. 1970. Effect of level of protein intake on urinary and fecal calcium and calcium retention in young adult males. J. Nutr. 100:1425.

Johnson, W. H., R. D. Goodrich and J. C. Meiske. 1971. Metabolism of radioactive sulfur from elemental sulfur, sodium sulfate and methionine by lambs. J. Anim. Sci. 32:778.

Jonsson, G. 1978. Milk fever prevention. Vet. Rec. 102:165.

Jonsson, G. and B. Pherson. 1970. Trials with prophylactic treatment of parturient paresis. Vet Rec. 87:575.

Jonsson, G and B. Pherson. 1975. The effect of two calcium levels in the dry period ration on the incidence of milk fever. Page 398 in Proc. 8th int. conf. Dis. Catt. Mibalo. Netherlands.

Jorgensen, N. A. 1974. Combating milk fever. J. Dairy Sci. 57:933.

Kendall, K. A., K. E. Harshbarger, R. L. Hays and E. E. Ormiston. 1969. Some responses of dairy cows to acid or alkaline dietary additives. J. Dairy Sci. 52:931.

Key, L. Y., D. Carnes, S. Cole, M. Holtrop, Z. Bar-Savit, F. Shapiro, R. Arceci, J. Steinberg, C. Gundberg, A. Kahn, S. Teitebaum and C. Anast. 1984. Treatment of congenital osteopetrosis with high dose calcitriol. New Engl. J. Med. 310:409.

Kichura, T. S., R. L. Horst, D. C. Beitz and E. T. Littledike. 1982. Relationship between prepartal dietary calcium and phosphorus, vitamin D metabolism and parturient paresis in dairy cows. J. Nutr. 112:480.

Kilderberg, P. 1968. Clinical acid-base physiology: studies of neonates, infants and young children. Williams and Wilkins, Baltimore. MD.

Kim, Y. and H. M. Linkswiler. 1979. Effect of level of protein intake on Ca metabolism and on parathyroid and renal function in adult male. J. Nutr. 109:1399.

Klahr, S. D. 1982. Disorders of acid-base metabolism. Chapter 4 in Disorders of mineral, water and acid-base metabolism. J. C. M. Chan and J. R. Gill, Jr. ed. John Wiley and sons, New York, NY.

Kronfeld, D. S., G. P. Mayer, and C. F. Ramberg, Jr. 1976. Calcium homeostasis in cattle. Page 169 in Handbook of Physiology and Endocrinology. Am. Physiol. Soc. Washington, DC.

Kronfeld, D. S. and C. F. Ramberg, Jr. 1970. The mammary drain of calcium. Page 107 in Parturient hypocalcemia. J. J. B. Anderson. ed. Academic Press, New York, NY.

Kronfeld, D. S. and C. F. Ramberg, Jr. 1971. Calcium kinetics in cows with hypomagnesemia. J. Dairy Sci. 54:794 (abs.).

Krook, L., L. Lutwak, K. McEntre, P. A. Henrikson, K. Braun and S. Roberts. 1971. Nutritional hypercalcitoninism in bulls. Cornell Vet. 61:625.

Lane, V. M. and A. M. Merritt. 1983. Reliability of single sample phosphorus fractional excretion determination as a measure of daily phosphorus renal clerance in equids. Am. J. Vet. Res. 44:500.

Larvor, P. and M. L. Labat. 1978. Magnesium deficiency on calcium metabolism in the rat. Ann. Biol. Anim. Biochim. biophys. 18:149.

Lawson, D. E. M. 1978. Biochemical response of the intestine to vitamin D. Page 190 in Calcium in biological systems. D. E. M. Lawson. ed. Plenum Press, New York, NY.

Leclerc, H. and E. Block. 1989. Effects of reducing dietary cation-anion balance for prepartum dairy cows with specific reference to hypocalcemis parturient paresis. Can. J. Anim. Sci. 69:411

Lee, S. W., J. Russell and L. Avioli. 1977. 25-hydroxycholecalciferol to 1,25 dihydroxycholecalciferol conversion impaired by systemic metabolic acidosis. Science. 195:994.

Lemann, J. Jr., J. R. Litzow and E. J. Lennon. 1966. The effects of chronic acid loads in normal man: further evidence for the participation of bone mineral in the defense against chronic metabolic acidosis. J. Clin. Invest. 45:1608.

Lemann, J. Jr., J. R. Litzow and E. J. Lennon. 1967. Studies of the mechanisms by which chronic acidosis augments urinary calcium excretion in man. J. Clin. Invest. 46:1318.

Lemann, J. Jr., and A. S. Relman. 1979. The relationship of sulfur metabolism to acid-base balance and electrolyte excretion: the effect of DL-methionine in normal men. J. Clin. Invest. 38:2215.

L'Estrange, J. L. and R. F. E. Axford. 1964. A study of magnesium and calcium metabolism in lactating ewes fed a semi-purified diet low in magnesium. J. Agric. Sci. 62:353.

Lin, S. L., and J. C. M. Chan. 1973. Urinary bicarbonate: a titrimetric method for determination. Clin. Biochem. 6:207.

Linkswiler, H. M., C. L. Joyce, and C. R. Anand. 1974. Calcium retention of adult males as affected by level of protein and of calcium intake. Trans. N.Y. Acad. Sci. Series II 36:333.

Linkswiler, H. M., M. B. Zemel, M. Hegsted and S. Schuette. 1981. Protein induced hypercalciuria. Fed. Proc. 40:2429.

Little, W. L. and N. L. Wright. 1925. The etiology of milk fever in cattle. Br. J. Exp. Path. 6:129.

Littledike, E. T. 1976. Relationship of milk secretion to hypocalcemia in the dairy cow. J. Dairy Sci. 59:1947.

Littledike, E. T. and R. L. Horst. 1982. Vitamin D_3 toxicity in dairy cows. J. Dairy Sci. 65:749.

Littledike, E. T., J. W. Young and D. C. Beitz. 1981. Common metabolic diseases of cattle: ketosis, milk fever, grass tetany and downer cow complex. J. Dairy Sci. 64:1465.

Littledike, E. T., J. W. Young and D. C. Beitz. 1983. Common metabolic diseases of cattle: ketosis, milk fever, grass tetany and Downer cow complex. J. Dairy Sci. 64:1465.

Lomba, F., G. Chauvaux, E. Teller, L. Lengele and V. Bienfet. 1978. Calcium digestibility in cows as influenced by the excess of alkaline ions over stable acid ions in their diets. Br. J. Nutr. 39:425.

Louitit, J. T. and N. W. Nisbet. 1982. The origin of osteoclasts. Immunology. 161:193.

Luick, J. R., J. M. Boda and M. Kleiber. 1957. Some biokinetic aspects of calcium metabolism in dairy cows. Am. J. Physiol. 189:483.

Lunquist, P., J. Martensson, B. Sorbo, and S. Ohman. 1980. Turbidimetry of inorganic sulfate, ester sulfate and total sulfur in urine. Clin. Chem. 26:1178.

Ma, R. S. W., and J. C. M. Chan. 1973. Endogenous sulfuric acid production: a method of measurement by extrapolation. Clin. Biochem. 6:82.

Margen, S., J-Y. Chu, N. A. Kaufman, and D. H. Calloway. 1974. Studies on calcium metabolism. I. The calciuretic effect of dietary protein. Am. J. Clin. Nutr. 27:584.

Marks, S. C. Jr. 1983. The origin of Osteoclasts. J. Oral Pathol. 12:226.

Marquardt, J. P., M. F. Holick, R. L. Horst, N. A. Jorgensen and H. F. Deluca. 1974. Efficiency of 1a-hydroxy vitamin D on prevention of parturient paresis. J. Dairy Sci. 57:606.

Marquardt, J. P., R. L. Horst and N. A. Jorgensen. 1977. Effect of parity on dry matter intake at parturition in dairy cows. J. Dairy Sci. 60:929.

Matsumoto, T., Fontaine, O and H. Rasmussen. 1981. Effect of 1,25-dihydroxyvitamin D_3 on phospholipid metabolism in chick duodenal mucosal cell. J. Biol. Chem. 256:3354.

Mayer, B. P., J. W. Blum and L. J. Deftos. 1975. Diminished prepartal plasma calcitonin concentrations in cows developing parturient hypocalcemia. Endocrinology. 96:1478.

Mayer, G. P., C. F. Ramberg, Jr, D. S. Kronfeld, R. M. buckle, L. M. Sherwood, G. D. Aurbach and J. T. Potts, Jr. 1969. Plasma parathyroid hormone concentration in hypocalcemic parturient cows. Am. J. Vet. Res. 30:1587.

McManus, J. and F. W. Heaton. 1969. The effect of magnesium deficiency on calcium homeostasis in the rat. Clin. Sci. 36:297.

McManus, J. and F. W. Heaton. 1970. The influence of magnesium on calcium release from bone in vitro. Biochim. Biophys. Acta. 215:361.

Mendel, L. B. and S. R. Benedict. 1909a. The paths of excretion for inorganic compounds. IV. The excretion of calcium. Am. J. Physiol. 25:1.

Mendel, L. B. and S. R. Benedict. 1909b. The paths of excretion for inorganic compounds. V. The excretion of calcium. Am. J. Physiol. 25:23.

Mongin, P. 1981. Recent advances in dietary anion-cation balance in poultry. Page 109 in Recent advances in animal nutrition-1981. W. Haresign. ed. Butterworths, London, UK.

Moodie, E. W. 1960. Some aspects of hypocalcemia in cattle. Vet. Rec. 72:1145.

Moodie, E. W. 1965. Modern trends in animal health and husbandry: Hypocalcemia and hypomagnesemia. Br. J. Vet. J. 121:338.

Moodie, E. W. and A. Robertson. 1961. Diretary intake of the parturient cow. Res. Vet. Sci. 2:217.

Moodie, E. W. and A. Robertson. 1962. Some aspects of calcium metabolism in the dairy cow. Res. Vet. Sci. 3:470.

Moore, C. W. 1970. Ionized calcium in normal ultrafiltrates and whole blood determined by ion exchange electrodes. J. Clin. Invest. 49:318.

Mundy, G. R. and L. G. Raisz. 1981. Disorders of bone resorption. Page 1 in Disorders of mineral metabolism. F. Bronner and J. W. Coburn. ed. Academic Press, New York, NY.

Murer, H. and B. Hildman. 1981. Transcellular transport of calcium and phosphate in the small intestinal epithelium. Am. J. Physiol. 240:G409.

National Research Council. 1989. Nutrient requirement of dairy cattle. 6th ed. Natl. Acad. Sci, Washington, DC.

Nichols, K. L. R. and R. L. Tannen. 1987. Renal excretion of divalent ions in response to chronic acidosis: evidence that systemic pH is not the controlling variable. J. Lab. Clin. Med. 109:27.

Niedermeier, R. P., V. R. Smith and C. K. Whitehair. 1949. Parturient paresis. III. A study of various blood constituents at parturition in mastectomized cows. J. Dairy Sci. 32:927.

Nordin, B. E. C. 1976. Page 186 in Calcium, phosphate and magnesium metabolism. Churchill-Livingstone, Edinburg.

O'Donnnell, J. M. and M. W. Smith. 1973. Uptake of calcium and magnesium by rat duodenal mucosa analysed by means of competing metals. J. Physiol. 229:733.

Oetzel, G. R., J. D. Olson, C. R. Curtis, and M. J. Fettman. 1988. Ammonium chloride and ammonium sulfate for prevention of parturient paresis in dairy cows. J. Dairy Sci. 71:3302.

Olson, E. B., H. F. DeLuca and J. T. Potts. 1972. Calcitonin induction of vitamin D induced intestinal calcium absorption. Endocrinology 90:151.

Olson, W. G., N. A. Jorgensen, L. H. Shultz and H. F. Deluca. 1973. 25-hydroxycolecalciferol (25-(OH)D_3). I. Treatment for parturient paresis. J. Dairy Sci. 56:885.

Papworth, D. G. and G. Patrick. 1970. The kinetics of influx of calcium and strontium into rat intestine in vitro. J. Physiol. 210:999.

Paquay, R., F. Lomba, A. Lousse, and V. Blenfet. 1968. Statistical research on the fate of dietary mineral elements in dry and lactating cows. I. Calcium. J. Agric. Sci. (Camb). 71:173.

Payne, J. M. 1964. The responses of cows to experimentally induced hypocalcemia. II. Chronic experimental hypocalcemia. Vet. Rec. 76:77.

Payne, J. M. 1968. Milk fever. Outlook on Agric. 5(6):266.

Perry, H. M., W. Skogan, and J. C. Chappel. 1987. Conditioned medium from osteoclast-like cells mediates parathyroid hormone induced bone resorption. Calcif Tissue Int. 40:298.

Petito, S. L. and J. L. Evans. 1984. Calcium status of the growing rat as affected by diet acidity, phosphate and protein. J. Nutr. 114:1049.

Pickard, D. W. 1975. An apparent reduction in the incidence of milk fever acchieved by regulation of the dietary intake of calcium and phosphorus. Br. Vet. J. 131:744.

Popovici, A., C. F. Geschichter, A. Reinovsky and M. Rubin. 1950. Experimental control of serum calcium levels in vivo. Proc. Soc. Exp. Biol. Med. 74:415.

Raisz, L. G. 1965. Bone resorption in tissue culture. Factors influencing the response to parathyroid hormone. J. Clin. Invest. 44:103.

Raisz, L. G. 1976. Mechanisms of bone resorption and calcium regulation. Page 117 in Handbook of Physiology. G. D. Aurbach. ed. Williams aand Wilkins, Baltimore, MD.

Raisz, L. G. and I. Niemann. 1967. Early effects of parathyroid hormone and thyrocalcitonin in organ culture. Nature (London) 214:486.

Raisz, L. G. and I. Niemann. 1968. Effect of phosphate calcium and magnesium on bone resorption and hormonal response in tissue culture. Endocrinology. 85:446.

Ramberg, C. F. Jr., E. K. Johnson, R. D. Fargo and D. S. Kronfeld. 1984. Calcium homeostasis in cows with special reference to parturient hypocalcemia. Am. J. Physiol. 246:R698.

Ramberg, C. F. Jr., D. S. Kronfeld and G. D. A. Wilson. 1975. Regulation of calcium metabolism in cattle during growth, gestation, lactation and changes in diet. Page 200 in Digestion and metabolism in the ruminant. I. W. McDonald and A. C. I. Warner. ed. Univ. New England publishing Unit, Armidale, Australia.

Ramberg, C. F. Jr., G. P. Mayer, D. S. Kronfeld, L. M. Sherwood and J. T. Potts, Jr. 1966. Parathyroid hormone responses in parturient cows. J. Dairy Sci. 49:717.

Ramberg, C. F. Jr., G. P. Mayer, D. S. Kronfield, G. D. Aurbach, L. M. Sherwood and J. T. Potts, Jr. 1967. Plasma calcium and parathyroid hormone responses to EDTA infusion in the cow. Am. J. Physiol. 213:878.

Ramberg, C. F. Jr., G. P. Mayer, D. S. Kronfeld, and J. T. Potts, Jr. 1975. Dietary calcium, calcium kinetics and plasma parathyroid hormone concentration in cows. J. Nutr. 106:671.

Rasmussen, H., T. Matsumoto, O. Fontaine and D. B. P. Goodman. 1982. Role of changes in membrane lipid structure in the action of 1,25-dihydroxyvitamin D_3. Federation Proc. 41:72.

Rayssiguir, Y., J. M. Garel, M. Davicco and J. P. Barlet. 1977. Parathyroid hormone and calcitonin in magnesium deficient calves. Horm. Met. Res. 9:438.

Reddy, C. R., J. W. Coburn, D. L. Hartenbower, R. M. Friedler, A. S. Brickman, S. G. Massry and J. Jowsey. 1973. Studies on mechanisms of hypocalcemia of magnesium depletion. J. Clin. Invest. 52:3000.

Revell, P. A. 1986. Pathology of bone. Springer-Verlag, Berlin, Germany.

Reynolds, M. 1970. Mammary circulatory and respiratory changes associated with parturition in goats. Page 97 in Parturient hypocalcemia. J. J. B. Anderson. ed. Academic Press, New York, NY.

Robertson, A., H. Paver, P. Barden and T. G. Marr. 1960. Fasting metabolism of the lactating cow. Res. Vet. Sci. 1:117.

Roche, C., C. Bellaton, D. Pansu and F. Bronner. 1984. Simultaneous induction of CaBP and active calcium transport in rat duodenum by 1,25-dihydroxyvitamin D_3. Page 307 in Epithelial calcium and phosphate tansport. F. Bronner and M. Peterelik. ed. Alan R. Liss Inc., New York, NY.

Rodan, G. A. and T. J. Martin. 1981. Role of osteoblasts in hormonal control of bone resorption - a hypothesis. Calcif. Tissue Int. 33:349.

Rowland, G. N., C. C. Capen, D. M. Young and H. E. Black. 1972. Micrographic evaluation of bone from cows with experimental hypervitaminosis D diet induced hypocalcemia and naturally occurring parturient paresis. Calcif. Tiss. Res. 9:179.

Rude, R. K. 1985. Skeletal adenylate cyclase: effect of Mg, Ca and PTH. Calcif. Tiss. Int. 37:318.

Sachs, M. A., A. Bar, R. Cohen, Y. Mazur, E. Mayer and S. Hurwitz. 1977. Use of 1a-hydroxycholecalciferol in prevention of bovine parturient paresis. Am. J. Vet. Res. 38:2039.

Sansom, B. F, R. Manston and M. J. Vagg. 1983. Magnesium and milk fever. Vet. Rec. 112:447.

Schachter, D and S. Kowarski. 1985. Vitamin D and the intestinal membrane calcium binding protein. Page 210 In Calcium in biological systems. R. P. Rubin. ed. Plenum Press, New York, NY.

Schachter, D. and S. M. Rosen. 1959. Active transport of Ca^{45} by the small intestine and its dependence on vitamin D. Am. J. Physiol. 196:357.

Schryver, H. F., D. W. Meakim, J. E. Lowe, J. Williams, L. V. Soderholm, and H. F. Hintz. 1987. Growth and calcium metabolism in horses fed varying levels of protein. Equine Vet. J. 19:280.

Schuette, S. A., H. Hegsted, M. B. Zemel and H. M. Linkswiler. 1981. Renal acid, urinary cyclic AMP, and hydroxyproline excretion as affected by level of protein, sulfur amino acid and phosphorus intake. J. Nutr. 110:305.

Schuette, S. A., M. B. Zemel, and H. M. Linkswiler. 1980. Studies on the mechanisms of protein-induced hypercalciuria in older men and women. J. Nutr. 110:305.

Sherman, H. V. 1920. Calcium requirement in men. J. Biol. Chem. 44:21.

Sherwood, L. M., G. P. Mayer, C. F. Ramberg, Jr., D. S. Kronfeld, C. D. Aurbach and J. T. Potts, Jr. 1968. Regulation of parathyroid hormone secretion: proportional control by calcium, lack of effect of phosphate. Endocrinology. 83:1043.

Snedecor, G. W. and W. G. Cochran. 1980. Statistical methods. Iowa State Univ. Press, Ames, IA.

Stacy, B. D. and B. W. Wilson. 1970. Acidosis and hypercalciuria: renal mechanisms affecting calcium, magnesium and sodium excretion in the sheep. J. Physiol. 210:549.

Termine, J. D. and A. S. Posner. 1966. Infrared analysis of rat bones: age dependency of amorphous and crystalline mineralfractions. Science. 153:1523.

Timet, D. and Z. Stojevic. 1982. Influence of magnesium ions on gastric absorption of calcium in cattle. Vt. ArKiv. 52(6):247.

Trummel, C. L., L. G. Raisz, J. S. Blunt and H. DeLuca. 1969. 25-hydroxycholecalciferol. Science. 163:1450.

Vaes, G. 1968. On the mechanism of bone resorption. J. Cell Biol. 39:676.

Vagg, M. J. and J. M. Payne. 1970. The effects of ammonium chloride induced acidosis on calcium metabolism in ruminants. Br. Vet. J. 126:531.

Van Corven, E. J. J. M. and C. H. Van Os. 1984. Distribution of ATP-dependent Ca-transport between villus and cript cells in rat duodenum. Page 295 in Epithelial calcium and phosphate transport. F. Bronner and M. Peterlik. ed. Alan R. Liss Inc., New York, NY.

Van de Braak, A. E., A. Th. van't Klooster, J. C. van Hal-van Gestel, and A. Malestein. 1984. Influence of stage of lactation and calcium level of the ration on mobilization rate of calcium and excretion of hydroxyproline in urine in dairy cows. Studies with Na_2EDTA infusions in monozygotic twins. Zbl. Vet. Med. A. 31:725.

Van de Braak, A. E., A. Th. van't Klooster and A. Malestein. 1986a. Effects of low and high calcium intake prepartum on calcium mobilization rate around parturition in dairy cows. Vet. Quarterly. 8:12.

Van de Braak, A. E., A. Th. van't Klooster and A. Malestein. 1986b. Influence of prepartum calcium intake on calcium mobilization rate around parturition in dairy cows fed at a high prepartum feeding level. Vet. Quarterly. 8:24.

Van de Braak, A. E., A. Th. van't Klooster and A. Malstein. 1987. Influence of a deficient supply of magnesium during the dry period on the rate of calcium mobilization by dairy cows at parturition. Res. Vet. Sci. 42:101.

Verdaris, J. N. and J. L. Evans. 1974. Early lactation mineral nutrition and milk production as affected by diet calcium and pH. J. Dairy Sci. 57:623.

Verdaris, J. N. and J. L. Evans. 1976. Diet calcium and pH versus mineral balance in Holstein cows 84 days pre- to 20 days postpartum. J. Dairy Sci. 59:127.

Wang, C. and D. K. Beede. 1990. Effects of supplemental protein on acid-base status and calcium metabolism of Jersey cows. J. Dairy Sci. (in press).

Wasserman, R. H. and F. A. Kallfelz. 1962. Vitamin D_3 and unidirectional calcium fluxes across the rachitic chick duodenum. Am. J. Physiol. 203:221.

Wasserman, R. H., H. J. Armbrecht, F. Shimura, S. Meyer and Chandler. 1984. Vitamin D influences multiple phases of the intestinal calcium transport systems. Page 307 in Epithelial calcium and phosphate transport. F. Bronner and M. Peterlik. ed. Alan R. Liss Inc., New York, NY.

Wasserman, R. H. and A. N. Taylor. 1969. Some aspects of the intestinal absorption of calcium with special reference to vitamin D.

Watchorn, E. and R. A. McCance. 1937. Subacute magnesium deficiency in rats. Biol. J. 31:1379.

Welsh, J., R. Schwartz and L. Krook. 1981. Bone pathology and parathyroid gland activity in hypocalcemic magnesium deficient chicks. J. Nutr. 111:514.

Whiting, S. J. and H. H. Draper. 1981. Protein induced Calciuria in diabetic rats. J. Nutr. 111:178.

Wiggers, K. D., D. K. Nelson and N. L. Jacobson. 1975. Prevention of parturient paresis by a low calcium diet prepartum: a field study. J. Dairy Sci. 58:430.

Wilson, A. A. 1964. Hypomagnesemia and magnesium metabolism. Vet. Rec. 76:1382.

Witter, F., P. Contreras, M. Miranda and Y. R. Leon. 1985. Prevention of milk fever in cows by oral administration of bone meal. Vet. Arg. II:700.

Wolfgang, T. and E. Ritz. 1985. Sulfur containing amino acids are a major determinant of urinary calcium. Mineral Electrolyte Metab. 11:137.

Yarrington, J. T., C. C. Capen, H. E. Black, R. Re, J. T. Potts, Jr. and W. B. Geho. 1976. Experimental parturient hypocalcemia in cows following prepartal chemical inhibition of bone resorption. Am. J. Path. 83:569.

Yarrington, J. T., C. C. Capen, H. C. Black and R. Re. 1977. Effects of a low calcium prepartal diet on calcium homeostatic mechanisms in the cow: morphologic and biochemic studies. J. Nutr. 107:2244.

Yuen, D. E., H. H. Drape and G. Trilok. 1984. Effect of dietary protein on Ca metabolism in man. Nutr. Abs. Rew. 54(6):447.

Zemel, M., S. Schuette, M. Hegsted and H. M. Linkswiler. 1979. Effect of level of protein and of phosphorus intake on urinary calcium and sodium in men. Fed. Proc. 38:872 (Abs).

Zemel, M. B., S. A. Schuette, M. Hegsted and H. M. Linkswiler. 1981. Role of the sulfur containing amino acids in protein-induced hypercalciuria in man. J. Nutr. 111:545.

BIOGRAPHICAL SKETCH

Changzheng Wang, was born on September 19, 1959, in Hubei Province, China. He managed to go through primary education and graduated from high school in 1977. After taking the first Open National University Entrance Examination in more than a decade, he was accepted into Central-China Agricultural University, Wuhan, China, and graduated with a degree in veterinary medicine in 1981. After working briefly in Beijing Agricultural University, he went to the University of Saskatchewan, Canada and graduated with a degree of M.S in animal science in 1985. He joined the Dairy Science Department at the University of Florida in 1986.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Dr. David K. Beede, Chair
Associate Professor of Dairy Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Dr. Lynn B. Bailey
Professor of Food Science and Human Nutrition

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Dr. Alfred M. Merritt, II
Professor of Veterinary Medicine

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Dr. William W. Thatcher
Graduate Research Professor of Dairy Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Dr. Charles J. Wilcox
Professor of Dairy Science

This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

December, 1990

Jack L. Fry

Dean, College of Agriculture

Dean, Graduate School